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SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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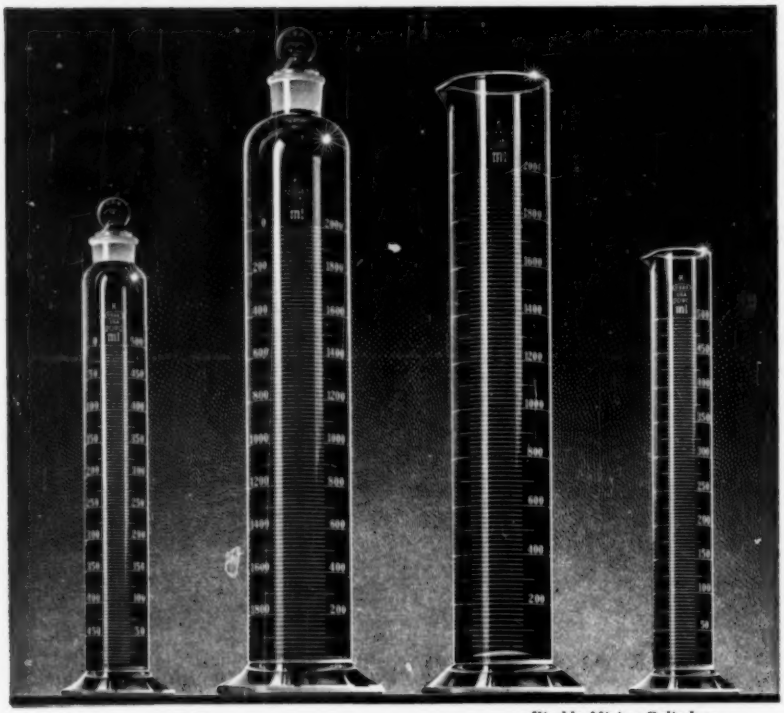

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The next annual meeting of the American Pharmaceutical Association will be held in Miami Beach, Florida, May 1 to 7, 1955. Members planning to present papers before any section during this meeting are earnestly requested and strongly urged to notify the appropriate Section secretary of their intention promptly. Titles and abstracts (not to exceed 200 words) of papers should be submitted by February 1, 1955, or preferably earlier, in order to insure a place on any section program. Send titles and abstracts of papers to:

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The above names, in accordance with paragraph 3 of the Procedure of the Selection of Recommended International Non-Proprietary Names for Drugs moving in International Commerce, was published in the *Chronicle of the World Health Organization* **8,** (October 1954). Comments on, or formal objections to, the above names may be filed within a period of six months from November 1, 1954, and should be forwarded to the Director-General, World Health Organization, Palais des Nations, Geneva, Switzerland.

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1955 Iodine Research Award Nominations Requested Prior to January 1, 1955

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1955 Chilean Iodine Educational Bureau, Inc. Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1955 Award, *nominations must be received on or before January 1, 1955.*

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

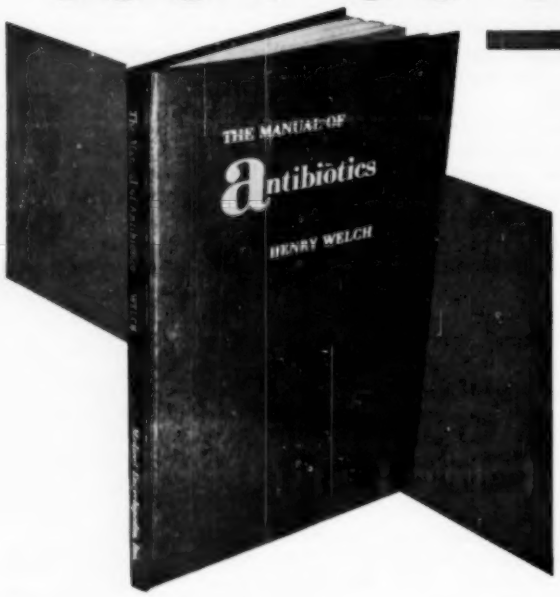
The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION's Council. The present committee includes Justin L. Powers, chairman; Paul Block, E. A. Brecht, Leroy D. Edwards, Harvey B. Haag, Lloyd M. Parks, and Heber W. Youngken.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its seventh year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Missouri, was the recipient of the fifth award. Dr. Jack Gross, Associate Professor of Anatomy, State University of New York, College of Medicine in Brooklyn received the sixth award which was conferred during the 1954 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Just Off the Press...

1954-55 MANUAL



For the first time, there are gathered between the covers of one book a list of the preparations, therapeutic index, generic and trade names, and names and addresses of producers of all existing antibiotics and their preparations.

This information, prepared under the editorial direction of Henry Welch, Ph.D., and published by the Medical Encyclopedia, Inc., is distributed exclusively by the American Pharmaceutical Association.

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To identify a trade name it is only necessary to check the trade name index. Opposite the trade name in question is the page number on which the generic term is given, along with the active ingredients and indications for the preparation. All other trade names that have been assigned to each preparation are also shown.

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(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

▶ In two insertions, **Abbott Laboratories** stresses the fast disintegration of *filmtab* **Erythrocin** stearate and emphasizes the "Iron-*plus*" content of **Iberol.**

▶ **Span, Tween, Arlacel** and **Sorbo** are just four of the **Atlas Powder Company's** many pharmaceutical aids.

▶ To provide rapid symptomatic relief in upper respiratory infections, **Hoffmann-La Roche Inc.** advocates **Thephorin-AC.**

▶ **Hynson, Westcott & Dunning** reports that **Nyloxin** ampules have proved highly effective in relieving pain in chronic arthritis.

▶ **Achromycin** (tetracycline **Lederle**) is described as approaching "*the* ideal broad-spectrum antibiotic, effective against . . . many mixed infections."

▶ **'Mi-Cebrin'** is advocated by **Eli Lilly and Company** as a dietary supplement "for people past forty."

▶ The following prescription specialties are listed by the **S. E. Massengill Company** in its announcement: **Adrenosem, Aminodrox, Broniacin, Livitamin, Massengill Powder, Obedrin, Salcedrox,** and **Semhyten.**

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▶ **Duraglas Rx Containers, Kimble** *Opticlear* vials and *Plastainer* (plastic) vials, reminds **Owens-Illinois,** enhance the dispenser's reputation.

▶ In its announcement, **Parke, Davis & Company** reiterates its rôle of service to the physician, the pharmacist and the public in dramatizing for the latter the importance of prompt and proper medical care.

▶ **Pfizer Laboratories** offers the reminder that its broad-spectrum tetracyclines—**Terramycin** and **Tetracyn**—are indicated whenever antibiotic therapy is required.

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▶ Its ready solubility in fruit juice, milk and infant formulas makes **Penalev (Sharp & Dohme)** "effective in all infections where oral penicillin is indicated."

▶ Two of **Smith, Kline & French Laboratories'** fast-moving items are **Benzedrex Inhalers,** effectively merchandised, and **Thorazine** for the control of nausea, agitation, and acute anxiety.

▶ **Rau-sed** for sedation and **Raudixin** for the treatment of hypertension constitute **Squibb's** insertions.

▶ **The Upjohn Company** affirms the efficacy of its antibiotic, **Panmycin,** and reaffirms the proven effectiveness of **Kaopectate** in treating diarrhea.

▶ Its oral hypotensive agent, **Methium,** and **—Peritrate—**for the prevention of attacks in angina pectoris, are the subjects of the **Warner-Chilcott** insertion.

▶ *New* **Thenfadil-S.P.C.** is described by **Winthrop-Stearns** as an effective cold remedy that will increase prescriptions.

Look for details in the Practical Pharmacy Edition of This Journal—Out November 20

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLIII — *NOVEMBER, 1954* — NUMBER 11 — CONSECUTIVE NO. 22

The Use of 2-Pipecoline in the Mannich Reaction*

By SAMUEL B. BRITTON, H. CECIL CALDWELL, and W. LEWIS NOBLES†

Mannich bases utilizing 2-pipecoline as the amine component have been prepared in an attempt to enhance the local anesthetic and/or antispasmodic action of the corresponding nonalkylated piperidine derivatives of this type.

CERTAIN substituted β-aminoketones and derivatives have been reported (1–4) to show both local anesthetic and antispasmodic activity. Such compounds as are here described may be prepared readily by means of the Mannich reaction:

$$RCOCH_3 + HCHO + R'_2NH \cdot HCl \rightarrow RCOCH_2CH_2NR'_2 \cdot HCl + H_2O$$

The extensive literature dealing with this reaction has been reviewed by Blicke (5).

Mannich and Lammering (1) reported that β-piperidinopropiophenone hydrochloride, a substance easily obtained from acetophenone, piperidine hydrochloride and paraformaldehyde, possesses local anesthetic activity. Blicke and Blake (2) discovered that the corresponding Mannich base, obtained from 2-acetylpyrrole, piperidine hydrochloride and paraformaldehyde, possesses marked activity of this type. Levvy and Nisbet (3) indicated that both the piperidino and dimethylamino Mannich bases derived from 2-acetylthiophene possess local anesthetic activity, though not so marked as that demonstrated by cocaine.

Denton and co-workers (4) in a series of papers described the antispasmodic activity of compounds of this type and their derivatives. It should be noted that, as was the case with the Mannich bases demonstrating local anesthetic activity, here also the most effective amino group from the standpoint of pharmacological activity is the piperidino group. Under the conditions reported by these workers for determining antispasmodic activity, β-piperidinopropiophenone hydrochloride and 2-(β-piperidinopropionyl)-thiophene hydrochloride possess antispasmodic activity equal to that of β-diethylaminoethyl diphenyl acetate hydrochloride (Trasentine®) and greater than that of papaverine.

Rider and Cook (6) noted that a slight increase in the purity of the piperidine used in the synthesis of piperidinopropanediol diphenylurethan hydrochloride (Diothane®) causes a marked increase in its local anesthetic activity. They reviewed the chemical and pharmacological properties of a group of compounds containing piperidine and 2-pipecoline (2-methylpiperidine), one of the contaminants present in commercial piperidine. Compounds of much lower physiological

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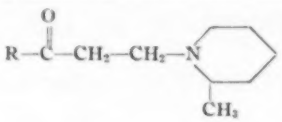
action were obtained in the propanediol series when piperidine containing either high or low boiling material was used in the synthesis. Consequently, they also studied the effect of the piperidine used on the activities of the benzoates and phenylurethans of the γ-substituted propanols. The piperidino derivatives are more active than their methylpiperidino homologs in the phenylurethan series. These workers confirmed the earlier observation of McElvain (7), however, that the reverse of this is true in the benzoate series, i.e., 2-methylpiperidinopropyl benzoate (Metycaine®) is more active than piperidinopropyl benzoate.

It is significant that the physiological activity of piperidine and methylpiperidine derivatives may differ widely. Thus, the authors became interested in preparing the 2-pipecoline analogs of the piperidino Mannich bases which had previously shown local anesthetic and/or antispasmodic activity. These analogs, as well as certain other keto bases, are listed in Table I. All the compounds listed were prepared by means of the Mannich reaction, employing paraformaldehyde, 2-pipecoline, and the appropriate ketone.

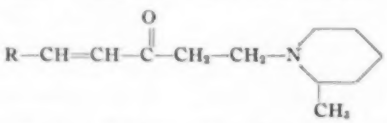
The aminoketones described in Table I are to be tested for both local anesthetic and antispasmodic activity.

Denton and co-workers (4) pointed out that the reduction of the piperidino Mannich bases to the corresponding aminoalcohols led to a marked increase in antispasmodic activity in all cases except the one involving a thiophene derivative. Also many of the tertiary alcohols, prepared by these workers by the addition of a Grignard reagent to the Mannich base, exhibited increased

TABLE I.—MANNICH BASES



No.	R	Formula	M. P.,[a] °C	Yield, %	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Chlorine Calcd.	Chlorine Found
1	Phenyl[b]	$C_{15}H_{21}NO \cdot HCl$	206–207	64	67.30	67.51	8.29	7.98		
2	*p*-Tolyl[b]	$C_{16}H_{23}NO \cdot HCl$	214–215	62	68.20	68.32	8.58	8.76		
3	3,4-Dimethylphenyl[b]	$C_{17}H_{25}NO \cdot HCl$	217–218	57	69.01	68.79	8.86	8.73		
4	4-Chlorophenyl[b]	$C_{15}H_{20}ClNO \cdot HCl$	161–162	38					11.73	11.58
5	4-Nitrophenyl[c]	$C_{15}H_{20}N_2O_3 \cdot HCl$	211–212	66	57.60	57.78	6.76	6.56		
6	4-Methoxyphenyl[b]	$C_{16}H_{23}NO_2 \cdot HCl$	210–211	69	64.54	64.72	8.12	8.08		
7	4-Ethoxyphenyl[b]	$C_{17}H_{25}NO_2 \cdot HCl$	205–206	56					11.36	11.49
8	2-Thienyl[d]	$C_{13}H_{19}NOS \cdot HCl$	204–205	51	57.23	56.71	7.39	6.98		
9	5-Chloro-2-thienyl[b]	$C_{13}H_{18}ClNOS \cdot HCl$	202–203	42	50.68	50.39	6.22	6.03		
10	5-Nitro-2-furyl[e]	$C_{13}H_{18}N_2O_4 \cdot HCl$	195–196	62	51.57	51.85	6.33	6.52		



11	Phenyl[f]	$C_{17}H_{23}NO \cdot HCl$	172–173	71	69.53	69.61	8.23	8.38		
12	2-Thienyl[g]	$C_{15}H_{21}NOS \cdot HCl$	195–196	48	60.08	60.26	7.40	7.29		
13	5-Chloro-2-thienyl[h]	$C_{15}H_{20}ClNOS \cdot HCl$	207–208	30	53.80	53.67	6.63	1.24		
14	2-Furyl[b]	$C_{15}H_{21}NO_2 \cdot HCL$–2	203–204	32	63.49	63.61	7.81	7.63		
15	5-Nitro-2-furyl[e]	$C_{15}H_{20}N_2O_4 \cdot HCL$	210–212	35	54.79	54.68	6.44	6.36		

[a] Melting points are uncorrected.
[b] Intermediate ketones from Distillation Products.
[c] Intermediate ketone obtained through the courtesy of Monsanto Chemical Company.
[d] 2-Acetothienone supplied through the courtesy of Jefferson Chemical Company.
[e] Intermediate ketones supplied through the courtesy of Eaton Laboratories.
[f] Benzalacetone supplied through the courtesy of S. B. Penick and Co.
[g] 4-(2-Thienyl)-3-buten-2-one prepared according to the method of H. Keskin, *et al.*, *J. Org. Chem.*, **16**, 199(1952).
[h] For ketones, see experimental section.

activity over that of the parent ketone. Work is currently under way to incorporate certain of these suggested modifications into the structures presented here; these studies will be reported in subsequent publications.

EXPERIMENTAL[1]

5-Chloro-2-acetylthiophene.—The following procedure is similar to the method employed by Mowry, Renoll, and Huber (8) for the preparation of the acetyl derivative of cyclohexylbenzene but differs in certain details. Hartough (9) points out that aluminum chloride polymerizes thiophene, therefore, in acylation reactions the order of addition of the reactants is quite important. Into a 2-L. flask surrounded by an ice-salt bath and equipped with a mechanical stirrer, dropping funnel, reflux condenser containing a calcium chloride drying tube, and thermometer was introduced 233 Gm. (1.75 moles) of anhydrous aluminum chloride and 1,000 cc. of carbon tetrachloride. The suspension was stirred rapidly and 125 cc. of acetyl chloride was added dropwise over a period of fifteen to twenty minutes. To this slurry was added 177 Gm. (1.5 moles) of 2-chlorothiophene[2] dropwise over a period of three hours, taking care that the temperature did not rise above 5°. Stirring was continued for one and one-half hours after the addition of the last of the 2-chlorothiophene. The reaction mixture was then poured with stirring into a 4-L. beaker containing ice, water, and hydrochloric acid. The mixture was then transferred to a separatory funnel, and the carbon tetrachloride solution was removed and washed successively with dilute hydrochloric acid and 10% sodium carbonate solution. It was then dried for twenty-four hours over anhydrous sodium sulfate. The carbon tetrachloride and unreacted 2-chlorothiophene were removed by distillation at atmospheric pressure. The residue in the distillation flask was cooled, and solidification ensued. The resulting pink mass was recrystallized from aqueous ethanol to yield 128 Gm. (58% yield) of a solid, m. p. 45–47°, reported (10) 46.5–47°.

4-(5-Chloro-2-thienyl)-3-buten-2-one.—5-Chloro-2-thenaldehyde, prepared in 40% yield from 2-chlorothiophene by the method of Campaigne and Archer (11), was condensed with acetone under the conditions of a typical Claisen-Schmidt reaction. Following the procedure of Keskin, *et al.*, (see footnote *h*—Table I), 40 Gm. (0.277 mole) of 5-chloro-2-thenaldehyde, 43 Gm. (0.74 mole) of acetone, 30 cc. of water, and 10 cc. of 10% sodium hydroxide solution gave 30 Gm. (59% yield) of a yellow viscous oil, b. p. 125–128° (1 mm.) which solidified on standing.

Anal.—Calcd. for C_8H_7ClSO: C, 51.47; H, 3.78. Found: C, 51.17; H, 3.59.

Mannich Bases (Table I).—The keto bases were prepared by the general procedure of dissolving 0.1 mole of 2-pipecoline in 20–25 cc. of absolute alcohol and then rendering the solution acid to litmus by the careful addition of concentrated hydrochloric acid. To this solution was added 0.1 mole of the ketone and 4.5 Gm. of paraformaldehyde. This mixture was heated to reflux for a period of two to three hours, after which the hot solution was poured into 100 cc. of acetone, cooled to room temperature, and then refrigerated until the crystalline product appeared. The solid thus obtained was removed by filtration and recrystallized from an ethanol-acetone solution.

In the case of the furan derivatives a slight modification in the above procedure proved necessary. The procedure of Levvy and Nisbet (3) for the preparation of Mannich bases from acetylfuran was employed after the alcoholic solution of the amine hydrochloride was prepared as described above.

[1] Carbon and hydrogen analyses by Weiler and Strauss, Oxford, England.

[2] Supplied through the courtesy of Socony-Vacuum.

SUMMARY

1. Fifteen Mannich bases containing 2-pipecoline as the amine moiety of the molecule have been prepared.

2. Ten of the ketones used in this study are acetophenone derivatives or simple aralkyl ketones of this type.

3. Five of the ketones are α,β-unsaturated ketones of the type represented by benzalacetone.

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(11) Campaigne, E., and Archer, W. L., *J. Am. Chem. Soc.*, **75**, 989(1953).

5-Dialkylamino-1-(2-thienyl)-1-penten-3-ones and Related Compounds*

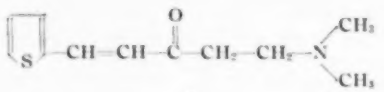
By SAMUEL B. BRITTON, H. CECIL CALDWELL, and W. LEWIS NOBLES†

Mannich bases from 4-(2-thienyl)-3-buten-2-one were prepared as vinylogs of similar compounds obtained from 2-acetothienone in an attempt to enhance the anesthetic and/or antispasmodic effect of the latter class of compounds.

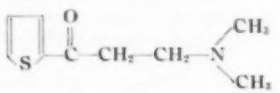
In view of the recent studies of others (1, 2) who have observed local anesthetic and/or antispasmodic activity in Mannich bases derived from 2-acetothienone, we became interested in learning whether or not vinylogs of these compounds would possess similar activity. Since the physical and chemical properties of a compound and its vinylog are markedly similar (3), further inquiry into the physiological activity of vinylogous substances appeared to be justified (4).

EXPERIMENTAL DESIGN

5-(4-Dimethylamino)-1-(2-thienyl)-1-penten-3-one (I), prepared from 4-(2-thienyl)-3-buten-2-one by means of the Mannich reaction, is a vinylog of 2-(β-dimethylaminopropionyl)-thiophene (II). Compound II, also readily prepared by the Mannich reaction (1), has been classed as a local anesthetic agent by Levvy and Nisbet and as a spasmolytic



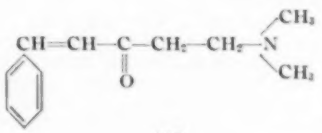
I



II

agent by Denton, *et al.* (2).

Furthermore, interest in this type of compound was increased as a result of the *in vitro* antibacterial activity recently reported by Burckhalter and Johnson (5) for 5-dimethylamino-1-phenyl-1-penten-3-one (III), which may be readily prepared from benzalacetone by means of the Mannich reaction.



III

Recently, the pharmacology of thiophene analogs of medicinally valuable benzene derivatives has been of special interest. The compounds listed in Table I are thiophene analogs of the benzalacetone Mannich bases mentioned above.

The amino ketones listed in Table I will be screened for their pharmacological activity.

TABLE I.—5-DIALKYLAMINO-1-(2-THIENYL)-1-PENTEN-3-ONES

No.	Amino group	M. P.,[a] °C.	Yield, %	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
1	Dimethylamino	172	65	$C_{11}H_{15}NOS\cdot HCl$	53.76	53.56	6.56	6.80
2	Diethylamino	100–101	49	$C_{13}H_{19}NOS\cdot HCl$	57.00	56.83	7.36	7.29
3	Diethanolamino[b]	107–108	21	$C_{13}H_{19}NO_3S\cdot HCl$	51.05	50.76	6.59	6.46
4	Dipropylamino[c]	124–125	51	$C_{15}H_{23}NOS\cdot HCl$	59.68	59.39	8.01	7.56
5	Diisopropylamino[c]	212–214	27	$C_{15}H_{23}NOS\cdot HCl$	59.68	60.07	8.01	8.16
6	4-Morpholinyl	172–173	68	$C_{13}H_{17}NO_2S\cdot HCl$	54.25	54.41	6.29	6.40
7	1-Pyrrolidyl[d]	169–170	30	$C_{13}H_{17}NOS\cdot HCl$	57.42	57.29	6.67	6.61
8	1-Piperidyl	189–190	68	$C_{14}H_{19}NOS\cdot HCl$	58.83	59.44	6.70	6.98
9	1-(2-Methylpiperidyl)	195–196	48	$C_{15}H_{21}NOS\cdot HCl$	60.08	60.26	7.40	7.29
10	Dibenzylamino	213	22	$C_{23}H_{23}NOS\cdot HCl$	69.41	69.74	6.08	6.01

[a] Melting points are uncorrected.
[b] Amine supplied through the courtesy of Carbide and Carbon Chemicals Co., New York, N. Y.
[c] Amines supplied through the courtesy of Sharples Chemicals Inc., Philadelphia, Pa.
[d] Amine supplied through the courtesy of E. I. Du Pont de Nemours & Co., Wilmington, Del.

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† Assistant Professor of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy, University of Mississippi.

DISCUSSION

These ten related keto bases were all prepared by means of the Mannich reaction, using paraformaldehyde, the desired secondary amine and 4-(2-thienyl)-3-buten-2-one.

The starting ketone was prepared by the Claisen-Schmidt method, following the procedure of Keskin, *et al.* (6). The 2-thenaldehyde used in the preparation of the butenone was prepared for the most part by the method of Campaigne and Archer (7). Also, the method of the direct formylation of thiophene by N-methylformanilide and phosphorus oxychloride as proposed by King and Nord (8) was employed to a limited extent. The use of dimethylformamide as suggested by Campaigne and Archer (7), in the case of 2-thenaldehyde, affords practically equivalent yields to that obtained with N-methylformanilide. Also, as is pointed out by these workers, dimethylformamide is relatively inexpensive as compared with N-methylformanilide.

In the preparation of 2-thenaldehyde by the method of Campaigne and Archer (7), considerable difficulty was experienced in getting the oily layer of aldehyde to separate from the reaction mixture. We were not able to note a point of neutralization, with subsequent separation of product, by the careful addition of a saturated solution of sodium acetate. However, when a solution containing the exact amount of sodium acetate needed to neutralize the hydrochloric acid generated in the reaction was added to the reaction mixture, separation of layers occurred and the aldehyde could be ether extracted. Yields of 65–70% were obtained consistently using this modification. Prior to this time it was necessary to steam-distill the reaction mixture, which would not separate into layers, in order to obtain the desired aldehyde. The yield of product thus obtained was significantly lower than the above figure.

EXPERIMENTAL PROCEDURE[1]

Procedure A.—The aminoketones were prepared by the general procedure of heating at reflux temperature 0.1 mole of the butenone with 0.1 mole of the amine hydrochloride and 4.5 Gm. of paraformaldehyde in 20–25 ml. of absolute alcohol containing two drops of concentrated hydrochloric acid. At the end of two hours, the solution was poured into 100 cc. of acetone and the mixture allowed to cool to room temperature. The mixture was then refrigerated until the solid product precipitated. The solid thus obtained was removed by filtration and recrystallized to analytical purity from a solution of alcohol-acetone.

Procedure B.—This method was utilized only when amines, not generally available as the crystalline hydrochlorides, were utilized. The secondary amine was dissolved in 20–25 cc. of alcohol and this solution was made slightly acidic to litmus by the dropwise addition of concentrated hydrochloric acid. To this alcoholic solution was added the ketone and paraformaldehyde and the general procedure of *A* was utilized from this point on.

SUMMARY

Ten Mannich bases derived from 4-(2-thienyl)-3-buten-2-one have been prepared using various dialkyl and heterocyclic secondary amines.

[1] Carbon and hydrogen analyses are by Weiler and Strauss Oxford, England.

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Possible Utilization of Polyelectrolytes in Enhancing Drug Absorption*

By TAKERU HIGUCHI, ROY KURAMOTO†, LLOYD KENNON‡, THOMAS L. FLANAGAN§, and ANDREW POLK§

Both theoretical considerations and *in vitro* studies indicate that the rate of absorption of ionic drugs may be enhanced by use of polyelectrolytes. It is shown that the rate of transfer of substances such as sodium salicylate, and potassium benzyl penicillin through cellophane membranes is materially increased by presence of such polyelectrolytes, as sodium carboxymethylcellulose, sodium polyacrylate, and others.

ALTHOUGH there is evidence that nondiffusible polyelectrolytes can associate with oppositely charged drug ions and thus retard their absorption, the converse phenomenon of enhancement of drug absorption by such ionic substances has not been fully recognized. This communication is concerned with the presentation of some theories which explain this effect and experimental data which demonstrate its relative magnitude.

THEORY

These phenomena are largely explicable on the basis of the Donnan Membrane Theory. For a system at equilibrium consisting of a solution of a sodium salt of a nondiffusible polyelectrolyte, NaR, contained in a semipermeable sac in contact with a solution of diffusible ions, e. g., sodium salicylate, the distribution of the latter will be unequal across the sac membrane. Thus the system will be:

$$\begin{array}{c|c} Na^+ & Na^+ \\ S^- & S^- \\ & R^- \end{array}$$

where:

Na^+ = the dissociated sodium ions.
S^- = the salicylate ions.
R^- = the dissociated nondiffusible macromolecule.

On the basis of the Donnan membrane theory, the following relationship can be derived for the systems of this type (1, 2):

$$\frac{c_o}{c_i} = \sqrt{1 + \frac{R^-}{c_i}} \qquad (1)$$

where:

c_o = concentration of the diffusible negatively charged ion in the "outside" solution in moles per liter.
c_i = concentration of the diffusible negatively charged ion in the "inside" solution in moles per liter.
R^- = concentration of the dissociated nondiffusible polyelectrolyte expressed in gram equivalents of charged group per liter.

In the derivation of the above equation, R^- was taken as the concentration of the dissociated portion of the polyelectrolyte, i. e., the number of effective charges on the macromolecules per liter divided by Avogadro's number. Many polyelectrolytes, because of the high electrical charge associated with each molecule, tend to bind the *gegen* ions rather strongly on to the molecules. In such instances only the residual unpaired electrical charges are effective in influencing the rate of distribution of the diffusing ions. Huizenga, *et al.* (3), for example, found by transference experiments that sodium polyacrylate was not completely dissociated even in dilute solutions. This is probably also true with sodium carboxymethylcellulose (CMC).

By use of a dialytic procedure and Eq. 1, it is possible to determine the relative degree of ionic dissociation of these polyelectrolytes. Thus, if a polyelectrolyte, as CMC, were completely dissociated, the effective concentration of CMC based on Eq. 1 would be equal to the equivalent concentration of the total CMC added. If these two variables are plotted, a slope of one would be obtained. If, however, all the carboxyl groups are not available due to association with cations, the slope will be less than one, the numerical value of the slope being a direct measure of the degree of dissociation.

If a second diffusible electrolyte, as sodium chloride, is present, the above equation must be modified to read:

$$\frac{c_o}{c_i} = \sqrt{1 + \frac{R^-}{c_i + c_{iCl^-}}} \qquad (2)$$

where c_{iCl^-} is the concentration of the negative ion of the electrolyte, i. e., the concentration of the chloride ion inside the sac. Or stated more generally:

$$\frac{c_o}{c_i} = \sqrt{1 + \frac{R^-}{\Sigma_i(c_i)_k}} \qquad (3)$$

where $\Sigma_i(c_i)_k$ is the total concentration of all the negatively charged species other than the polyelectrolyte.

It is evident according to the above equations that the equilibrium concentration of a negatively

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‡ Fellow of American Foundation for Pharmaceutical Education.
§ Smith, Kline and French Laboratories.

charged ion (drug or otherwise) inside any semipermeable sac would be strongly influenced by the presence of negatively charged polyelectrolytes especially if the negative ion concentration is relatively low. It is apparent, furthermore, that an analogous relationship can be derived for positively charged ions, the equation governing this being quite similar to Eq. 1.

From a practical drug administration standpoint, we are not concerned usually with equilibrium situations as discussed above, but rather with the kinetics of the system, that is, the rates at which these drugs are transported through the sac membrane. Although one can expect such rates of ionic drug absorptica to be enhanced by the presence of like charged nondiffusible ions, rigorous and complete thermodynamic development of such a kinetic problem is extremely difficult. Nevertheless, it seems reasonable to expect that the rate of such a permeation reaction may be roughly proportional to the relationship expressed in Eq. 1 except that the corresponding rates are substituted for the concentration:

$$\frac{\text{Rate in presence of } PE}{\text{Rate in absence of } PE} = \sqrt{1 + \frac{(R^-)}{(S^-)}} \quad (4)$$

where:

(S^-) = initial concentration of the diffusible anion in moles per liter.

(R^-) = Concentration of the dissociated nondiffusible polyelectrolyte expressed in gram equivalents of charged group per liter.

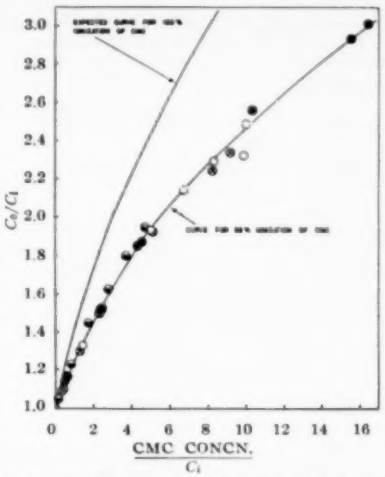

Fig. 1.—A plot demonstrating the exclusion effect of sodium carboxymethylcellulose on salicylate and penicillin ions at 0° C. The ratio of the concentration of the drugs outside and inside the cellophane bags, C_o/C_i, at equilibrium has been plotted against the concentration of the polyelectrolyte inside the bags divided by the concentration of the drugs inside the bags. The different drug concentrations refer to the initial concentrations. ●, Sodium salicylate, 3.12×10^{-3} M./L. ○, Sodium salicylate, 6.24×10^{-3} M./L. ◒, Sodium salicylate, 12.5×10^{-3} M./L. ⊗, Benzyl penicillin, 5.37×10 M./L.

EXPERIMENTAL RESULTS

Equilibrium Studies.—Experimental investigations were carried out to determine the extent of the postulated exclusion effect on negatively charged ions, e. g., salicylate, penicillin, etc., by the negatively charged polyelectrolytes, sodium carboxymethylcellulose, sodium polyacrylate, and the alginates. In these studies the experimental procedure previously employed for other studies on the binding effects of macromolecular substances on drugs was used (4). The method consisted essentially of bringing two solutions, one containing the macromolecular component and the other the drug under study, into equilibrium across a semipermeable membrane. The membrane was so chosen that it permitted the low molecular weight drug to pass freely through the barrier and achieve equilibrium in both solution phases, yet at the same time such that it acted as an impermeable barrier toward the polymeric substance.

The experimental data obtained demonstrating the exclusion effect of the added CMC on salicylate and penicillin ions are shown in Fig. 1. The results have been expressed as the fraction c_o/c_i as a function of the ratio (total CMC)/c_i where c_o represents the concentration of the diffusible negatively charged ion in the "outside" solution and c_i represents the concentration of the diffusible negatively charged ion in the "inside" solution. The upper curve represents theoretical values predicted by Eq. 1 and based on the assumption that CMC was totally dissociated. The lower curve represents the experimental values obtained. Two factors may be responsible for the disagreement between the two curves; (*a*) the system does not adhere strictly to Eq. 1, or (*b*) the CMC is not completely dissociated in solution as discussed above. From other studies made with polyelectrolytes, the latter appears to be the more satisfactory explanation. Thus, if the CMC is assumed to be only 53% dissociated the theoretical values would be in agreement with the experimental values and the two curves will coincide exactly.

A similar deviation from the theoretical values is obtained for the exclusion effect of sodium polyacrylate on salicylate and phthalate ions as shown in Fig. 2. In this case, if the polyelectrolyte concentration is assumed to be only 14% dissociated, the theoretical curve will coincide with the experimental curves.

The dependency of the exclusion effect on the polyelectrolyte concentration is more evident in Fig. 3 where the ratio of the salicylate concentration in the "outside" and "inside" solutions has been plotted as a function of the total polyelectrolyte concentration. It is apparent that the presence of a negatively charged polyelectrolyte has a strong influence on the equilibrium concentration of a negatively charged ion especially if the negative ion concentration is relatively low.

The relative proportion of the polyelectrolyte in the form of an uncharged associated complex can be evaluated from the preceding data if it is assumed that these systems adhere strictly to the Donnan equilibrium relationship. Equation 1 is more conveniently used for this purpose in the following form:

$$CMC^- = \frac{(S^-)_o^2 - (S^-)_o^2}{(S^-)_i} \qquad (5)$$

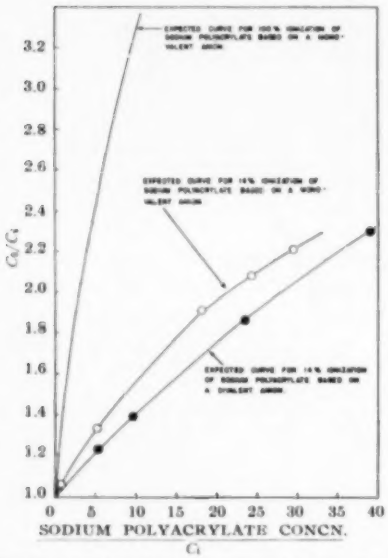
EXPECTED CURVE FOR 100% IONIZATION OF SODIUM POLYACRYLATE BASED ON A MONOVALENT ANION
EXPECTED CURVE FOR 14% IONIZATION OF SODIUM POLYACRYLATE BASED ON A MONOVALENT ANION
EXPECTED CURVE FOR 14% IONIZATION OF SODIUM POLYACRYLATE BASED ON A DIVALENT ANION
C_o/C_i
SODIUM POLYACRYLATE CONCN.
C_i

Fig. 2.—A plot showing the exclusion effect of sodium polyacrylate on salicylate and phthalate ions. (See Fig. 1.) ○, Sodium salicylate, 6.23 × 10^{-3} M./L. ●, Potassium phthalate, 4.13 × 10^{-3} M./L.

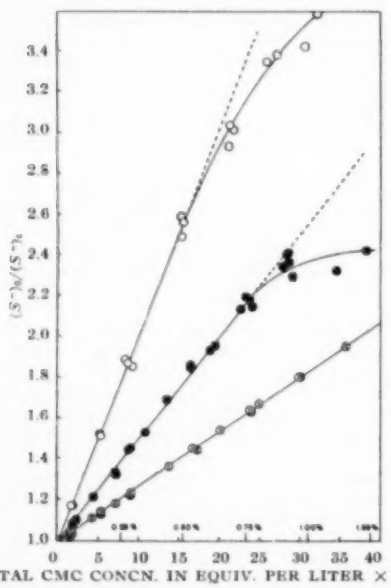

Fig. 3.—Graph demonstrating the dependency of the exclusion effect on the polyelectrolyte concentration at various salicylate ion concentrations. (See Fig. 1.) ○, 3.12 × 10^{-3} M./L. ●, 6.24 × 10^{-3}. ⊗, 12.5 × 10^{-3}.

where CMC^- refers to the dissociated and, therefore, effective polyelectrolyte concentration expressed in gram equivalents of charged group per liter and where (o) and (i) refer to the "outside" and "inside" solutions. The experimental results for the salicylate and penicillin systems expressed in this manner are shown in Figs. 4–7.

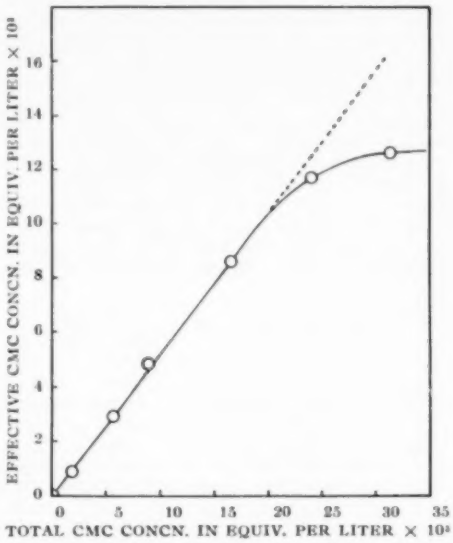

Fig. 4.—Interaction between sodium carboxymethylcellulose and benzyl penicillin where the slope is a direct measure of the degree of dissociation of CMC. The effective concentrations were determined on the basis of Donnon equilibrium calculations. Concn. = 5.37 × 10^{-3} M./L.

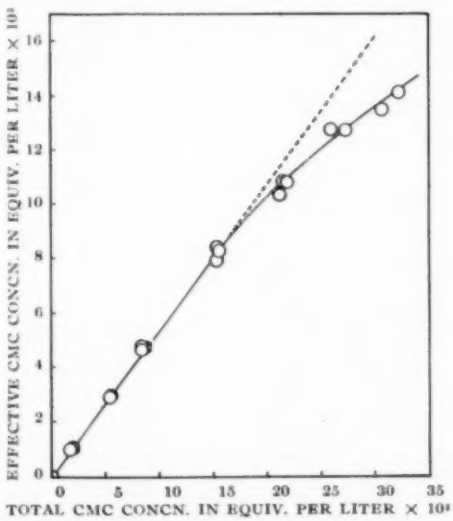

Fig. 5.—A plot showing the interaction between CMC and sodium salicylate at 0° C. as a means of determining the degree of dissociation of CMC. (See Fig. 4.) Concn. = 3.12 × 10^{-3} M./L.

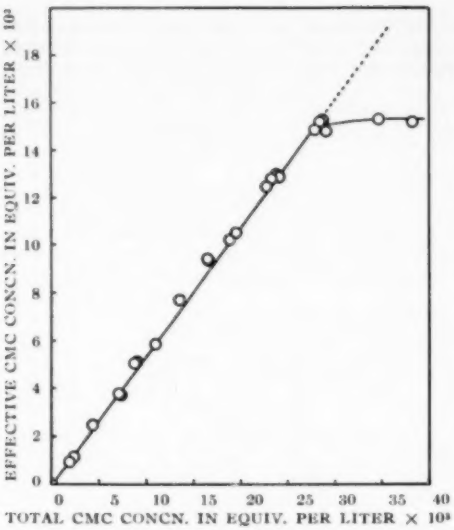

Fig. 6.—A graph illustrating the degree of dissociation of CMC with a higher salicylate concentration. (See Fig. 4.) Concn. = 6.25×10^{-3} M./L.

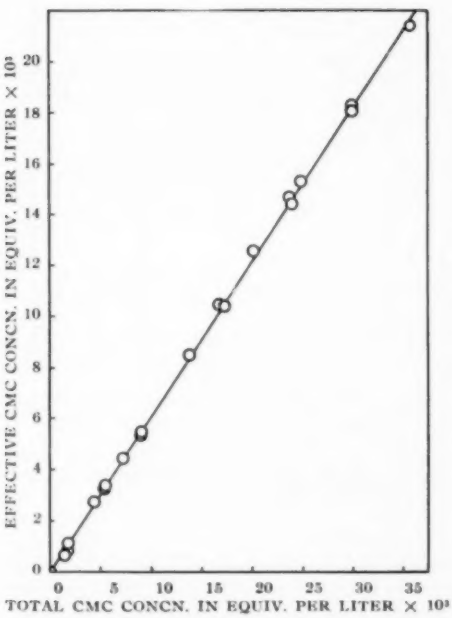

Fig. 7.—Interaction between sodium carboxymethylcellulose and sodium salicylate at a salicylate concentration of 12.5×10^{-3} M./L. (See Fig. 4.) Concn. = 12.5×10^{-3} M./L.

These results suggest that a part of the CMC is associated with the sodium or potassium ions and that corresponding carboxyl groups are not available to exert their influence on the ionic distribution. The numerical value of the slope is a direct measure of the degree of dissociation. From the slope of the lines it is apparent that approximately 47% of the total sodium CMC added to these systems is associated with the cations and exerts no exclusion effect.

A direct measure of the degree of dissociation of other polyelectrolytes, e.g., sodium polyacrylate and the alginates, can also be obtained by expressing the experimental data according to an equation that is essentially the same as Eq. 5. The results expressed in this manner for the salicylate systems are shown in Figs. 8 and 9. For a divalent anion such as the phthalate a slightly different equation can be easily derived from the Donnan Membrane Theory (2). Data for the phthalate system are shown in Fig. 10.

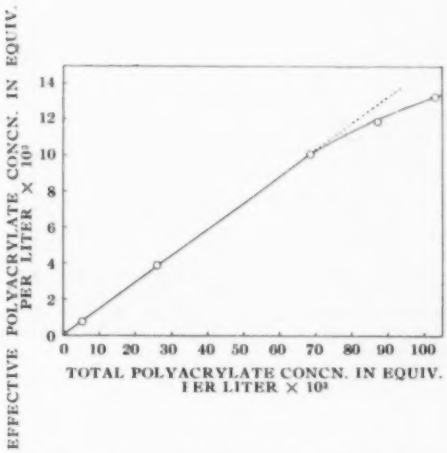
EFFECTIVE POLYACRYLATE CONCN. IN EQUIV. PER LITER × 10³
0 2 4 6 8 10 12 14
0 10 20 30 40 50 60 70 80 90 100
TOTAL POLYACRYLATE CONCN. IN EQUIV. PER LITER × 10³

Fig. 8.—A plot showing the interaction between sodium salicylate and sodium polyacrylate where the slope is a measure of the degree of dissociation of sodium polyacrylate. (See Fig. 4.) Concn. = 6.23×10^{-3} Eq./L.

From the slope of the lines it is apparent that polyacrylate and the alginate are also only partly dissociated. In fact, on the basis of the theory, 86% of the total polyacrylate and 56% of the alginate added are associated with the cations and exerts no exclusion effect.

Kinetic Studies.—The enhancement of the absolute rate of drug diffusion of negatively charged ions, such as scarlet red sulfonate and salicylate, by the presence of a negatively charged nondiffusible polyelectrolyte was investigated. The influence of the polyelectrolyte, sodium CMC, on the rate of diffusion of these ionic substances from semipermeable sacs were followed by placing the bags of these solutions in bottles containing distilled water and determining the amount of material eluted out from each sac as a function of time. Control runs were made simultaneously without the polyelectrolyte to determine the normal rate of diffusion. The bottles were placed during both runs in an ice chest and the whole assembly was shaken during

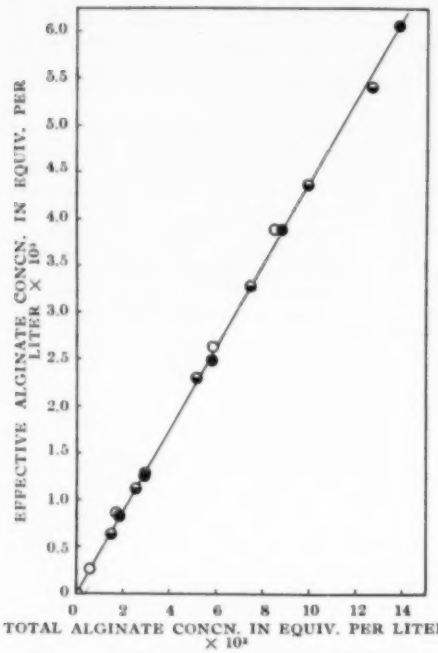

Fig. 9.—The interactions between salicylates and the corresponding alginates. (See Fig. 4.) ◒, Sodium salicylate, 6.25×10^{-3} M./L. ●, Potassium salicylate, 7.24×10^{-3} M./L. ○, Ammonium salicylate, 7.25×10^{-3} M./L.

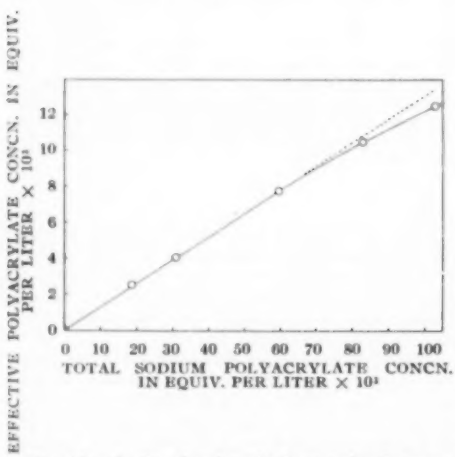

Fig. 10.—A plot showing the interaction between potassium phthalate and sodium polyacrylate at 0° C. where the macromolecule is only 14% dissociated. (See Fig. 4.) Concn. = 4.13×10^{-3} M./L.

the extraction period. Since the membranes used were not identical, a number of runs were made simultaneously and the values were averaged to obtain statistically reliable data.

The influence of the polyelectrolyte, sodium CMC, on the rate of diffusion of scarlet red out of cellophane bags is seen in Fig. 11, where the concentrations of the external solution has been plotted directly as functions of time. The plots are essentially straight lines because these systems were still far removed from equilibrium at the end of the runs, the slow rate of diffusion being due to the large size of the dye molecules. It is evident that the polyelectrolyte had more than doubled the rate of transfer of the dye across the barrier. In obtaining these plots initial dye concentration of 4.12×10^{-3} moles per liter and initial CMC concentration of 2.00×10^{-2} equivalents per liter were employed.

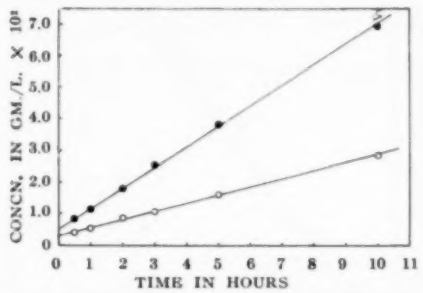

Fig. 11.—Graph showing the rate of diffusion of Biebrich Scarlet Red from cellophane bags in the presence and absence of sodium carboxymethylcellulose. ○, Blank. ●, With CMC.

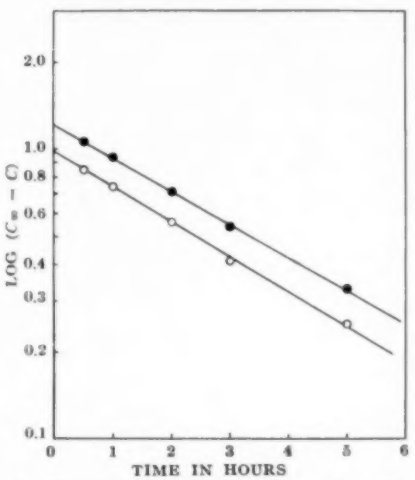

Fig. 12.—A plot of the amount of sodium salicylate diffusing from cellophane bags in presence and absence of sodium carboxymethylcellulose as a function of time at 0° C. ○, Blank. ●, With CMC. Influence of negatively charged polyelectrolytes of sodium salicylate.

The exclusion effect of polyelectrolyte on more readily diffusible drug ions represented by salicylate

ion is evident in Fig. 12. Here, to permit better comparison, log $C_\infty - C_t$ has been plotted against time where C_∞ is the external concentration at equilibrium and C_t is the external concentration at any time t. Such plots yield straight lines since diffusional process obeys the first order rate law. For these systems, the presence of the polyelectrolyte seems to promote the rate of the diffusional process to about the same extent as it influences the final equilibrium distribution. Thus the addition of the polyelectrolyte to the internal solution effectively increased its concentration with respect to the drug as predicted by Eq. 1 as far as the diffusional process was concerned.

Earlier workers, by *in vivo* experiments, have obtained results which are in qualitative agreement with the present theory. Sullivan, *et al.* (5), investigating the effect of phosphonic cation exchange resins on rats, have shown that these resins tend to repel anions from the intestinal tract into the blood stream, and thereby increase the anionic urinary content. Goldschmidt and Dayton (6), in experiments with dogs, have found that sulfate ions appear to drive chloride ions rapidly from colons of the animal, the sulfate ion here acting as the nondiffusible electrolyte.

Burns and Visscher (7) have extended these observations by comparing the behavior of anions other than sulfate. They found that phosphate strongly forces the chloride from the intestinal lumen and acetate does the same to a smaller extent. These *in vivo* experiments are thus in apparent agreement with theories presented above, the nondiffusible inorganic ions behaving in a manner similar to the polyelectrolytes.

EXPERIMENTAL

Reagents.—*Sodium carboxymethylcellulose* (CMC-70 Low Viscosity, Hercules Powder Company). *Sodium polyacrylate* (XF-133, Rohm and Haas Company). *Sodium alginate* (Kelcosol®, Kelco Company). *Alginic acid* (Kelacid®, Kelco Company). *Penicillin G Potassium Salt*, crystalline. *Sodium salicylate*, U. S. P. *Potassium phthalate*. *Biebrich Scarlet* (water-soluble, National Aniline Division, Allied Chemical and Dye Corporation).

Method of Analysis.—A Beckman spectrophotometer, Model DU, was used in the analysis of the internal and external solutions of the following substances at the dilution and wavelength values listed: sodium salicylate, 1:50 dilution, 296 mμ; benzyl penicillin, 1:5 dilution, 270 mμ; potassium salicylate, 1:50 dilution, 296 mμ; ammonium salicylate, 1:50 dilution, 296 mμ; Biebrich Scarlet (Scarlet red sulfonate), diluted as required, 355 mμ.

Dialysis Studies.—The method employed has been described elsewhere (4).

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Vitamin B_{12} Studies—The Instability of Vitamin B_{12} in the Presence of Thiamine and Niacinamide*

By MILTON BLITZ, EDWARD EIGEN, and EPHRAIM GUNSBERG

Vitamin B_{12} is not stable in a B-complex solution. The destruction of vitamin B_{12} takes place in the presence of thiamine and niacinamide in combination. Experimental data are presented to confirm this effect.

A SURVEY of the current literature indicates that vitamin B_{12} should be stable in a solution containing other B-complex factors (1, 2). However, our laboratories found vitamin B_{12} unstable in various B-complex injectable products currently on the market. The composition of four of these is shown in Table I.

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Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

Room temperature stability studies of vitamin B_{12} in the above B-complex products show vitamin B_{12} to be very unstable (Table II). In fact, practically all the vitamin B_{12} was lost in products *B* and *D* after only three months' storage at room temperature. These products were purchased on the open market and their age prior to receipt at the laboratory is not known.

EXPERIMENTAL

A study was initiated to determine which factor(s) was responsible for the destruction of vitamin B_{12} in B-complex parenterals. The formula of

TABLE I.—COMPOSITION OF VARIOUS B-COMPLEX PARENTERALS

Ingredient	Product A	B	C	D
Vitamin B_{12}, μg./cc.	5	30	20	5
Thiamine hydrochloride, mg./cc.	25	25	10	50
Riboflavin, mg./cc.	2	2	3	1
Niacinamide, mg./cc.	100	100	50	50
Pyridoxine hydrochloride, mg./cc.	5	5	5	5
Panthenol, mg./cc.	5	5	5	5
Phenol, %	0.5	...	...	..
Benzyl alcohol, %	...	2	...	..
Gentisic acid ethanolamide, %	...	...	2.5	1

TABLE II.—THE STABILITY OF VITAMIN B_{12} IN B-COMPLEX INJECTABLE SOLUTIONS STORED AT ROOM TEMPERATURE

B-Complex Injectable	B_{12} Label Claim, μg./cc.	Length of Time Stored at Room Temperature	Vitamin B_{12} Activity,[a] μg./cc.
A	5	Initial	4.50
		3 months	2.40
B	30	Initial	None found at 5 μg./cc. level
		3 months	0.07
C	20	Initial	16.0
		3 months	11.5
D	5	Initial	5.25
		3 months	None found at 5 μg./cc. level
		3 months	None found at 1 μg./cc. level
D	5	Initial	5.00
		2 months	None found at 2.5 μg./cc. level
		9 weeks	None found at 0.2 μg./cc. level

[a] Measured by the microbiological procedure described in the U. S. Pharmacopeia, Fourteenth Revision, Third Supplement, Mack Publishing Co., Easton, Pa., 1950.

product *D* was used for this purpose. The *p*H of this solution was 4.25, an optimum *p*H for preparations containing vitamin B_{12}. The simple elevated temperature test proposed by Gakenheimer (1) was used to test the compatibility of the various components with vitamin B_{12}. This consists of heating the sample for four hours at 100°.

Preliminary studies were conducted on the preservative, gentisic acid ethanolamide (also used to help solubilize the riboflavin), and the rubber stoppers used in preparing the vials. Both of these might possibly have been a cause for the destruction of vitamin B_{12} (2). Tests showed that this was not the case since no significant losses of the vitamin were observed in tests at the elevated temperature.

Since these two factors had been eliminated as destructive agents the other components of the injectable solution, namely the vitamins, were examined for their effect.

Various B-complex solutions were prepared with the complete formula of product *D* (Table I) adjusted to *p*H 4.25, and the same with one vitamin omitted at a time. The results of this experiment, shown in Table III, indicate that vitamin B_{12} is unstable in the presence of thiamine and niacinamide in combination.

TABLE III.—COMPATIBILITY OF CRYSTALLINE VITAMIN B_{12} AND B-COMPLEX FACTORS

Test Solution, B-Complex Injectable	Loss of Vitamin B_{12} After Heating at 100° for 4 Hours, %
Complete formula[a]	>83
Without thiamine	10
Without riboflavin	>83
Without niacinamide	5.9
Without pyridoxine	>83
Without panthenol	>83

[a] Product *D*, Table I.

These results were obtained whether the vials were prepared in air or under nitrogen.

Confirmation of the above data was obtained in experiments using crystalline vitamin B_{12}, thiamine hydrochloride, and niacinamide only. The same results were obtained with different batches of thiamine hydrochloride and niacinamide and when thiamine mononitrate and niacin were used.

Experiments were then conducted to determine the effect of various concentrations of niacinamide alone (Table IV) and thiamine alone (Table V) in solutions adjusted to *p*H 4.25.

TABLE IV.—EFFECT OF VARYING AMOUNTS OF NIACINAMIDE ON A SOLUTION CONTAINING 5μg./cc. OF VITAMIN B_{12}

Niacinamide, mg./cc.	Loss, %, of Vitamin B_{12} After Heating at 100° C. for 4 Hours	Storage at 45° C. 5 Days	Storage at 45° C. 3 Weeks
1	6.7	None[a]	None[a]
5	7.0	None[a]	None[a]
10	7.4	None[a]	None[a]
25	14.3	None[a]	None[a]
50	14.0	None[a]	None[a]
75	14.3	None[a]	None[a]
100	12.4	None[a]	None[a]

[a] Losses of 5% or less were considered insignificant since the microbiological method is accurate to ±10%.

TABLE V.—EFFECT OF VARYING AMOUNTS OF THIAMINE HYDROCHLORIDE ON A SOLUTION CONTAINING 5μg./cc. OF VITAMIN B_{12}

Thiamine Hydrochloride, mg./cc.	Loss, %, of Vitamin B_{12} After Heating at 100° for 4 Hours	Storage at 45° 5 Days	Storage at 45° 3 Weeks
1	20	None[a]	None[a]
5	28	None[a]	None[a]
10	28	None[a]	None[a]
25	24	None[a]	None[a]
50	44	None[a]	None[a]
75	52	None[a]	None[a]
100	56	None[a]	None[a]

[a] Losses of 5% or less were considered insignificant since the microbiological method is accurate to ±10%.

It was found that vitamin B_{12} is stable in the presence of relatively large quantities of niacinamide at 100° for four hours, that is, within the experimental limits of this test (1). However, the destruction of

vitamin B_{12} was significant at 100° for four hours in the presence of higher concentrations of thiamine.

When vitamin B_{12} was found incompatible with thiamine and niacinamide at 100° for four hours, all the stability studies were repeated with the storage conditions at 45° for five days and for three weeks for further confirmation of results. The 100° temperature while considered excellent for positive compatibility, may be too drastic and decomposition products formed at this temperature may cause destruction of vitamin B_{12}. The five-day 45° storage test has been employed by other investigators and found reliable for stability studies of vitamin B_{12} (3).

TABLE VI.—EFFECT OF VARYING AMOUNTS OF BOTH THIAMINE HYDROCHLORIDE AND NIACINAMIDE ON A SOLUTION CONTAINING 5μg./cc. OF VITAMIN B_{12}

Concentration of Both Thiamine Hydrochloride and Niacinamide,[a] mg./cc.	Loss, %, of Vitamin B_{12} After Heating at 100° C. for 4 Hours	Storage at 45° C. 5 Days	Storage at 45° C. 3 Weeks
1	45.5	None[b]	27.3
5	>93	None[b]	34.6
10	>95	9.6	44.4
25	>95	16.4	72.7
50	>95	47.9	95.0
75	>95	71.4	>97
100	>95	84.6	>99

[a] Each solution contained equal amounts of thiamine hydrochloride and niacinamide and was adjusted to *p*H 4.25.
[b] Losses of 5% or less were considered insignificant since the microbiological method is accurate to ±10%.

The results of these experiments indicate that vitamin B_{12} is stable in the presence of niacinamide alone at 45° (Table IV) and thiamine alone at 45° (Table V). However, vitamin B_{12} was found to be unstable in the presence of both thiamine and niacinamide at 45° for five days and three weeks in solutions at *p*H 4.25 (Table VI). Tests at 100° for four hours are included for comparison.

As little as 1 mg./cc. each of thiamine and niacinamide is sufficient to destroy 45.5% of the vitamin B_{12} activity after heating at 100° for four hours. These quantities destroy 27.3% of the vitamin after three weeks at 45°. At the 5 mg./cc. level, destruction is greater than 95% at 100° for four hours and 34.6% after three weeks at 45°. Increasing the concentrations of thiamine and niacinamide increases the destruction of vitamin B_{12} so that at the 100 mg./cc. level, destruction is 84.6% complete after five days at 45°, and greater than 99% after three weeks at this temperature.

SUMMARY

1. Vitamin B_{12} is not stable in a B-complex solution at *p*H 4.25 containing thiamine and niacinamide among its constituents.

2. Oxidation appears not to be a factor in the decomposition of vitamin B_{12} in the B-complex solution.

3. Large losses of vitamin B_{12} were found to be a function of the concentration of both thiamine and niacinamide in the solution.

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Pharmacological Properties of Two New Antihistaminics of Prolonged Action*

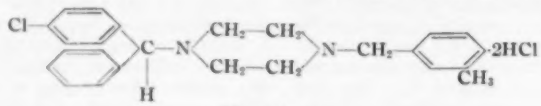
By S. Y. P'AN, J. F. GARDOCKI, and J. C. REILLY

A study was made of the pharmacological action of two new antihistaminic compounds of prolonged action. These compounds contain, besides piperazine, three benzylic rings. Both compounds, meclizine (*p*-chlorbenzhydryl-*m*-methyl-benzyl piperazine dihydrochloride) and buclizine (1-*p*-chlorbenzhydryl- 4-*p*- tertiary-butyl-benzyl piperazine dihydrochloride), show marked protective activity against nebulized histamine and lethal doses of intravenously injected histamine in guinea pigs. The onset of these actions is relatively slow but the duration of action is much more prolonged. The acute toxicity in mice and chronic toxicity in dogs and rats of both compounds indicate that they are relatively nontoxic.

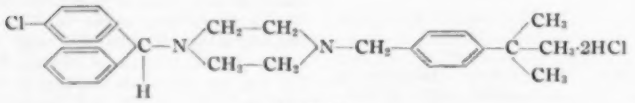
THERE HAVE BEEN in recent years a great number of synthetic compounds prepared as histamine antagonists. Morren, *et al.* (1), Grivsky (2), and Verdonk (3) reported the synthesis of a new class of antihistaminic compounds containing, besides the diethylene diamine (piperazine), three benzylic rings. These new compounds are characterized by exceptional prolonged antihistaminic potency. The most

* Received March 10, 1954, from the Research Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.
The authors are very much indebted to Miss Doris Jaeger and Mrs. Patricia Oldford for technical assistance.

promising members of this group are (I) 1-*p*-chlorbenzhydryl-*m*-methylbenzylpiperazine dihydrochloride,[1] and (II) 1-*p*-chlorbenzhydryl-4-*p*-tertiary butylbenzylpiperazine dihydrochloride.[2]



Meclizine I



Buclizine

We have given these compounds the generic names meclizine[3] and buclizine,[4] respectively. The present communication presents detailed pharmacological results obtained with these compounds.

EXPERIMENTAL METHOD

The following methods were employed for the evaluation of antihistaminic activity of these compounds. Comparative data for other antihistaminics such as tripelennamine, diphenhydramine and chlorocyclizine are included.

Protection Against Lethal Doses of Intravenously Injected Histamine in Guinea Pigs.—Antihistamine compounds were administered orally or intravenously to groups of guinea pigs weighing 300–400 Gm. and 1, 3, 5, 24, or 72, etc., hours later were given intravenously 10 MLD (MLD of histamine in guinea pigs was found to be 0.5 mg./Kg.) of histamine and the number of animals protected by the antihistaminic against histamine was recorded.

Prevention of Bronchospasm from Nebulized Histamine in Guinea Pigs.—Guinea pigs, one at a time, were confined in a glass chamber and exposed to aerosolized 0.2% solution of histamine dihydrochloride at 1, 3, 5, 17, 24, 72, etc., hours (Table I) after oral administration of test compounds. Only those guinea pigs suffering asphyxial collapse within four minutes were employed for the test. Any guinea pig surviving six minutes' exposure to nebulized histamine was classified as protected and such protection was attributed to the drug.

Action on Blood Pressure.—Female cats were anesthetized with Dial® (70 mg./Kg., intraperitoneally) and prepared for blood pressure recording from the carotid artery by means of a mercury manometer. Following control response to 1 μg. of histamine diphosphate or acetylcholine hydrochloride, one of the antihistaminics at a dose of 3 mg./Kg. was given intravenously. Ten minutes later a dose of histamine or acetylcholine was repeated and the vasodepressor responses were compared to those obtained prior to the injection of the antihistaminic.

Protection Against Histamine-induced Spasm of Isolated Strips of Guinea-Pig Ileum.—Small segments of guinea-pig ileum were suspended in a 50 ml. bath of Tyrode's solution at 39°. The concentration of the antihistaminic to abolish spasm induced by a concentration of 0.02 μg. (1:5,000,000) of histamine diphosphate per ml. of perfusion fluid was determined.

Acute Toxicity.—The acute toxicity of meclizine and buclizine was determined by intraperitoneal and oral routes in male albino Swiss mice weighing from 17 to 23 Gm. The LD_{50} and 95% confidence limits were determined according to the method of Litchfield and Wilcoxon (4).

Chronic Toxicity.—Chronic tolerance studies are in progress in dogs and rats. The drug is given to dogs orally, six times a week, by stomach tube. Rats are fed a diet containing graded concentrations of meclizine or buclizine.

RESULTS AND DISCUSSION

Protection Against Lethal Doses of Histamine and Histamine Aerosol—*in Vivo.*—The data on the protective action of meclizine, buclizine, tripelennamine, and chlorocyclizine against histamine aerosol and intravenous injection of lethal doses of histamine are presented in Table I. The effectiveness of meclizine and buclizine by mouth and their unusually prolonged duration of action as compared to those of tripelennamine and chlorocyclizine are apparent. Whereas the protective actions of tripelennamine and chlorocyclizine disappear within three and seventeen hours, respectively, those of meclizine and buclizine last for a much longer period of time. For instance, in protective tests against lethal doses of intravenous histamine, buclizine was able to protect 100% of guinea pigs against 10 MLD of histamine up to five days and 90% on the seventh day. Meclizine was able to protect 100 and 66% of guinea pigs at the end of 24 and 72 hours, respectively.

One additional characteristic of buclizine, as seen from these data, is its relatively slow onset as compared to that of tripelennamine and chlorocyclizine. The significance of this feature will be discussed later.

The results obtained on the protective action of these new antihistaminics against nebulized histamine are also in close agreement with the former test, i. e., relatively slow onset and unusually prolonged duration of action.

[1] This compound has been studied under the Union Chemique Belge S.A. number U.C.B. 5062 or Postafene.
[2] This compound has been studied under the Union Chemique Belge S.A. number U.C.B. 4445.
[3] Supplied as Bonamine® Brand meclizine by Chas. Pfizer & Co., Inc.
[4] Supplied as Vibazine® Brand buclizine by Chas. Pfizer & Co., Inc.

TABLE I.—PROTECTIVE ACTIVITIES OF PIPERAZINE DERIVATIVES AGAINST HISTAMINE AEROSOL AND INTRAVENOUSLY INJECTED HISTAMINE IN GUINEA PIGS

Compound	Oral Dosage, mg./Kg.	No. of Guinea Pigs	Per Cent of Guinea Pigs Protected At																	
			1 Hr.		3 Hr.		5 Hr.		17 Hr.		1 Day		2 Days		3 Days		4 Days		5 Days		6 Days		7 Days	
			A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]	A[a]	IV[b]
Meclizine	16	42	47	50	87	100	100	100	100	..	..	100	100	50	60	16	66	0	0	0	0	33	0	0	..
Buclizine	2.5	43	75	0	20	100	86	100	100	..	..	100	100	100	100	100	100	100	100	66	100	50	82	50	90
Tripelennamine	10	49	30	80	83	10	25	0	14	..	..	0	0	..	..	0	..	0	..	..	0	..	..	..	..
Chlorocyclizine	16	30	..	83	..	100	..	83	..	20	..	0	..	..	..	..	..	..	..	..	..	..	..	..	..

[a] A = Against histamine aerosol, 0.2% histamine dihydrochloride.
[b] IV = Against intravenously injected histamine, 10 MLD (MLD = 0.5 mg./Kg.).

TABLE II.—EFFECT OF ANTIHISTAMINE ON DEPRESSOR ACTION OF HISTAMINE AND ACETYLCHOLINE

Compound	Depressor Responses to Histamine, 1 μg./Kg.			Depressor Responses to Acetylcholine, 1 μg./Kg.		
	Before, mm. Hg	After, mm. Hg	Drop, %	Before, mm. Hg	After, mm. Hg	Drop, %
Meclizine	46	1.5	96.8	39	29	25.6
Buclizine	46	13	71.7	30	23	23.3
Tripelennamine	41	6	85.3	48	42	12.5
Diphenhydramine	52	2	96.1	55	63	0

Action on Blood Pressure.—Table II presents quantitative data on the effect of these new piperazine derivatives on the depressor effect of histamine and acetylcholine in cats (average of three cats for each compound). At a dose of 3 mg./Kg., both meclizine and buclizine were effective in blocking the vasodepressor response to histamine. They also exhibit slight blocking action against acetylcholine. The duration of their blocking action is considerably longer than with either tripelennamine or diphenhydramine.

Inhibition of Histamine-induced Spasm of Guinea-Pig Ileum.—Meclizine and buclizine have relatively weak *in vitro* activity in inhibiting the spasmogenic action of histamine on isolated guinea-pig ileum. The minimal effective concentrations of meclizine and buclizine in the bath are 1 and 10 μg./ml., respectively. (The minimal effective concentrations of tripelennamine and diphenhydramine are 0.02 and 0.01 μg./ml., respectively.) Such phenomena, together with the observation that the onset of *in vivo* protective action of these compounds against nebulized or intravenously injected histamine is slow, seem to point to the possibility that these compounds, especially buclizine, are split up into active compounds after absorption.

Toxicity.—*Acute Toxicity.*—The acute oral and intraperitoneal toxicities of meclizine and buclizine are given in Table III.

TABLE III.—ACUTE ORAL AND INTRAPERITONEAL TOXICITY OF MECLIZINE AND BUCLIZINE IN MICE

Compound	LD₅₀ (95% Confidence Limits)	
	Oral, mg./Kg.	Intraperitoneal, mg./Kg.
Meclizine	1600 (1330–2046)	625 (463–884)
Buclizine	2100 (1824–2394)	430 (344–538)
Tripelennamine	235 (204–270)	67 (57–79)
Diphenhydramine	...	60 (50–70)
Chlorocyclizine	500 (433–625)	95 (64–122)

In mice dying from toxic doses of either meclizine or buclizine, the earliest symptoms and signs are those of respiratory distress followed by excitement, convulsions, and death. Both compounds are considerably less toxic than tripelennamine, diphenhydramine, and chlorocyclizine.

Chronic Toxicity in Dogs.—Twenty-five female mongrel dogs were divided at random into groups of three or four dogs each and are receiving daily, six days a week, oral doses of meclizine or buclizine by stomach tube. An additional group of dogs serves as controls. During a period of six months, no abnormal symptoms or changes in behavior of the animals were noted. The study is now in its seventh month. Hematological examination (including red and white blood cell counts, hemoglobin content, cell volume, prothrombin time), and biochemical blood studies, (including blood sugar, nonprotein nitrogen, urea nitrogen, total proteins, albumin globulin and sodium, potassium, and chloride determinations), liver function tests (bromsulfphthalein), kidney function tests (phenolsulfonphthalein), and

urinalyses were made at monthly intervals and were all found to be within the normal range of variation.

Chronic Toxicity in Rats.—Four groups of twelve male albino rats, each weighing from 50 to 65 Gm. at the start of the experiment, are fed a diet of pulverized Rockland Farm rat ration containing graded concentrations of meclizine or buclizine (5, 10, 25, and 50 mg. %). Control rats are fed the basic diet. All rats are allowed water and diet *ad lib.* Daily food and water intake are recorded and animals are weighed at weekly intervals. The study is now in the seventh month. During a six-month period, all animals showed weight gain similar to that of the control rats. The difference in daily food consumption between the controls and each group of experimental animals during the same period was not significant for any group.

SUMMARY

1. Both meclizine and buclizine show marked protective activity against nebulized histamine and lethal doses of intravenously injected histamine in guinea pigs.

2. The onset of each of these actions with meclizine and buclizine is relatively slow but the duration of action is much more prolonged.

3. Meclizine has very weak and buclizine has no antagonistic action *in vitro* against histamine-induced spasm of guinea-pig ileum.

4. The acute toxicity in mice and chronic toxicity determinations in dogs and rats of both compounds indicate that they are relatively nontoxic.

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Analysis of Barbiturates—Comparison of Aqueous and Nonaqueous Methods*

By JOHN C. RYAN,† LEO K. YANOWSKI,† and CHARLES W. PIFER‡

A comparison between aqueous and nonaqueous methods of analysis shows that nonaqueous titrations are applicable to the analysis of barbiturates and prove equivalent to the official method in accuracy and precision as well as being less tedious and time consuming. The authors recommend the use of lithium methoxide as a titrant. The end points are sharpened slightly when a high ratio of benzene to solvent is employed. Thymol blue is found to be a satisfactory indicator and titration to a deep blue color gives the true end point. The Schmall extractor lends itself readily to nonaqueous analysis.

THIS STUDY was undertaken to provide a comparison between aqueous methods of analysis of barbiturates, as found in *The Pharmacopoeia of the United States* (3) and *New and Nonofficial Remedies* (2), and the nonaqueous methods of analysis. Since Fritz (1) and Vespe and Fritz (7) have shown the feasibility of titrating barbiturates in basic solvents, it was of interest to determine the accuracy and precision of this method as contrasted to the present official aqueous methods. Salts of the barbiturates as well as those tablets and capsules commonly prescribed are included, since the analysis of these forms sometimes presents difficulties due to the presence of diluents and excipients. It was felt at first that the salts could be dissolved in glacial acetic acid and titrated with standard perchloric acid. However, practically any other salt which would be present would also titrate and therefore lead to erroneous results. This method could be used in the case of a sample which contained only the barbiturate and its salt. Here the perchlorate titration would measure the amount of barbiturate salt and a lithium methoxide titration in dimethylformamide (D. M. F.) would measure the free barbiturate.

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The aid and assistance of Peter J. Conroy of the College of Pharmacy, Fordham University, in matters pertaining to official methods of analysis, is gratefully acknowledged.

Generally, a great number of individual manipulations are necessary in the aqueous methods. It was felt that extraction followed by titration would simplify the procedures, as well as lead to comparable accuracy and net saving in time required to perform the analysis.

EXPERIMENTAL

Apparatus.—50 ml. buret, automatic, graduated in 0.1 ml., magnetic stirrer, Squibb separator, Schmall extractor, for solvents lighter than water (6).

Reagents.—*Lithium methoxide*, 0.1 *N* (4). Dissolve 0.6 Gm. of freshly cut lithium metal in 150 ml. absolute methanol, cooling the flask during the addition. When the reaction is complete, add 850 ml. dry benzene. When cloudiness or precipitation occurs, add a sufficient amount of absolute methanol to clarify the solution. It is desirable to keep the volume of methanol at a minimum. Store the reagent in the reservoir of an automatic buret, protected from carbon dioxide and moisture.

Dimethylformamide, (D. M. F.), DuPont, technical grade.

Thymol blue indicator, 1.0% in D. M. F.

Standardization.—Accurately weigh out approximately 0.5 Gm. of benzoic acid (National Bureau of Standards), and dissolve in 10 ml. D. M. F. Perform the titration visually, using 3 to 5 drops of thymol blue as the indicator, the end point being taken as a dark blue color. A blank is run to correct for acidic impurities in the D. M. F. and usually is of the order of 0.10 ml. of titrant.

Titration.—*Free Acids.*—Accurately weigh out approximately 3 ml. of the corresponding barbiturate into a 250-meg. Erlenmeyer flask and dissolve in 10 ml. D. M. F. Add five drops of thymol blue and titrate with standard 0.1 *N* lithium methoxide to the appearance of a dark blue color. Excessive swirling of the solution should be avoided. In order to avoid the interference of atmospheric carbon dioxide, a stream of nitrogen may be directed over the surface of the solution during the titration. The authors have found that a magnetic stirrer gives sufficient agitation without carbon dioxide interference.

Sodium Salts, Tablets, and Capsules.—Depending upon the content of the barbiturate, a sample large enough to contain 3 to 4 meq. of the active ingredient is weighed into a Schmall extractor or a Squibb separator. Bring the sample into solution using 10 ml. 0.1 *N* NaOH. Acidify the solution using 15 ml. 0.1 *N* H_2SO_4, to liberate the free barbiturate. Extract the solution, the solvent depending upon the type of extractor used. For the Squibb separator, use seven 20-ml. portions of chloroform, and for the Schmall extractor, use 150 ml. of ether. Evaporate the extract on a steam bath, dissolve the residue in 10 ml. D. M. F., and titrate in the same way as the free acid.

The extract from the Squibb separator should be filtered through cotton, so as to remove water and any solid material which might slip through. When using the Schmall extractor, the drying tube, packed with a plug of cotton and anhydrous sodium sulfate is attached. The drying tube effectively removes the water in the organic phase.

RESULTS

Precision.—A series of analyses were performed in order to determine the precision of the nonaqueous method. Benzoic acid was used for the standardization, and three barbiturates were titrated for precision, using in each case 10 samples of the material. Using the maximum deviation of any result from the mean as a measure of the precision, the results are given in Table I.

From the table, it can be seen that it is possible to titrate barbiturates with a high degree of precision. Because of these encouraging results, it was decided to compare the aqueous methods with the nonaqueous methods. In all subsequent work, samples were analyzed in triplicate and the precision again is the maximum deviation from the mean

Bulk Barbiturates.—The compounds in Table II were analyzed either according to the procedure of *The Pharmacopeia of the United States, XIV* (3), by which the samples were extracted with chloroform and the residue weighed after evaporation of the solvent, or the method of *New and Nonofficial Remedies* (2), which involved solution of the sample in neutral ethanol and titration with standard base using thymolphthalein as the indicator. The compounds were also analyzed by the nonaqueous method of titration. The parent substances, barbituric acid and 2-thiobarbituric acid

TABLE I.—PRECISION

	Normality of Lithium Methoxide	Barbital, %	Phenobarbital, %	Aprobarbital, %
	0.1233	98.8	99.9	99.1
	0.1233	99.0	99.9	99.1
	0.1233	99.0	99.9	99.2
	0.1233	99.1	99.9	99.3
	0.1233	99.1	100.0	99.3
	0.1233	99.1	100.0	99.3
	0.1233	99.2	100.0	99.4
	0.1233	99.2	100.1	99.4
	0.1232	99.2	100.2	99.4
	0.1232	99.3	100.2	99.6
Average	0.1233	99.1	100.0	99.3
Precision	1/1,000	3/1,000	2/1,000	3/1,000

TABLE II.—ANALYSIS OF BULK BARBITURATES

Compound	By Aqueous Method, %	Precision	By Non-Aqueous Method, %	Precision
Amobarbital,[a] 5-ethyl-5-isoamyl barbituric acid	99.8	1/1,000	99.7	1/1,000
Pentobarbital,[a] 5-ethyl-5-(1-methylbutyl) barbituric acid	99.8	1/1,000	99.6	1/1,000
Thiopental,[a] 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid	99.9	1/1,000	100.0	1/1,000
Butethal,[b] 5-butyl-5-ethyl barbituric acid	99.7	1/1,000	99.6	4/1,000
Diallylbarbituric acid,[b] 5-5-diallylbarbituric acid	99.9	1/1,000	100.0	1/1,000
Hexobarbital,[b] 5-(1-cyclohexene-1-yl)-1,5-dimethyl barbituric acid	99.6	2/1,000	99.9	1/1,000
Mephobarbital,[b] 5-ethyl-1-methyl-5-phenyl barbituric acid	99.2	2/1,000	99.1	1/1,000
Barbituric acid	100.8	4/1,000	99.9	4/1,000
2-Thiobarbituric acid	101.2	1/1,000	100.5	2/1,000

[a] U. S. P. XIV.
[b] N. N. R. (1952).

TABLE III.—ANALYSIS OF BARBITURATE SALTS

Compound	Aqueous %	Method Precision	Nonaqueous Residue %	Titration Precision	Nonaqueous Extract %	Titration Precision
Amobarbital[a] sodium	100.0	1/1,000	99.9	2/1,000	100.0	1/1,000
Pentobarbital[a] sodium	99.1	0/1,000	98.8	1/1,000	98.7	1/1,000
Thiopental[a] sodium	93.3	1/1,000	93.3	0/1,000	93.4	1/1,000
Secobarbital[a] sodium, sodium 5-allyl-5-(1-methylbutyl)barbituric acid	99.9	0/1,000	99.9	1/1,000	100.0	1/1,000
Butethal[b] sodium	94.8	1/1,000	94.7	1/1,000	94.8	2/1,000
Cyclobarbital[b] calcium, calcium 5-(1-cyclohexene-1-yl)-5-ethyl barbituric acid	99.2	3/1,000	98.6	5/1,000	98.6	2/1,000

[a] U. S. P. XIV.
[b] N.N.R. (1952).

TABLE IV.—ANALYSIS OF DOSAGE FORMS OF BARBITURATES

Compound	Aqueous %	Aqueous Precision	Nonaqueous Direct Titration %	Direct Titration Precision	Nonaqueous Residue Titration %	Residue Titration Precision	Nonaqueous Extract Titration %	Extract Titration Precision
Amobarbital[a] tablets	62.0	2/1,000	62.1	2/1,000	62.1	2/1,000	62.2	2/1,000
Amobarbital[a] sodium capsules	105.6	4/1,000			105.3	4/1,000	105.0	2/1,000
Butethal[b] tablets	99.2	2/1,000	99.5	2/1,000	99.6	1/1,000	99.6	3/1,000

[a] U. S. P. XIV.
[b] N. N. R. (1952).

TABLE V.—COMPARISON BETWEEN OFFICIAL AND NONAQUEOUS PROCEDURES

	Operation	Aqueous, U. S. P.	Nonaqueous Schmall Extractor
1	Grind tablet mass	x	x
2	Weigh sample	x	x
3	Solution in base	x	x
4	Liberation by acid	x	x
5	Extraction	x (minimum of six hand extractions)	x (automatic)
6	Prepare tared vessel	x	..
7	Evaporate solvent	x	x
8	Ether addition and evaporation	x	..
9	Dry residue	x (2 hr)	..
10	Weigh residue	x	..
11	Titrate residue	..	x

were included, though these compounds are not of particular pharmaceutical importance.

It is of interest to note that the analysis of 2-thiobarbituric acid did not give reliable results by either method. However, when phenolphthalein is used as the indicator in the titrations in neutral ethanol, reproducible results are obtained, and titration to the first green shade of thymol blue instead of the customary deep blue color gave valid results in nonaqueous solvents.

Barbiturate Salts.—The results of the analyses of salts of the barbiturates are given in Table III. The aqueous results are by either of the official methods, the U. S. P. using extraction in the Squibb separator, evaporation of the extract, weighing the residue or subsequent titration of the residue in neutral ethanol according to the N. N. R. The titrations of the residues were performed upon the residues obtained from the official methods after Squibb extraction, and the extract titrations were performed upon samples extracted in the Schmall extractor.

Dosage Forms.—The data on the analyses of some of the typical dosage forms of the barbiturates are

given in Table IV. The methods are the same outlined in discussion of salts of the barbiturates with the additional modification of direct nonaqueous titration of the samples without previous extraction where possible. The results, in accordance with pharmaceutical convention, are expressed as the percentage of the labeled amount. In the case of amobarbital tablets, however, this convention could not be followed, since the sample was a combination of tablets of various sizes.

DISCUSSION

The experimental data show that nonaqueous titrations are applicable to the analysis of barbiturates. In addition to proving equivalent to the official methods in accuracy and precision, the nonaqueous method offers the advantage of being far less tedious and time consuming. Table V illustrates the time-saving features of the nonaqueous method involving the Schmall extractor as against a typical analysis by the methods of the U. S. P. In this table the *X* indicates that this particular operation must be performed in the analysis.

The authors recommend the use of lithium methoxide as a titrant because the use of sodium or potassium methoxide often leads to the formation of precipitates during the titrations. It was noted that when sodium methoxide was standardized according to the procedure outlined, there was formed a viscous gel, which obscured the end point and gave nonreproducible results. Lithium methoxide gave a precipitate with but one compound, barbituric acid. Even here, the precipitate was of a fine texture and settled rapidly, causing no interference. In this work, small volumes of dimethylformamide were used, though previous workers (1) called for much larger amounts. The small volumes tend to favor, as much as possible, a high ratio of benzene to solvent at the end point. In accordance with a previous publication (5), the end points are sharpened to a slight extent when a high ratio of benzene to solvent is employed. Thymol blue was found to be a satisfactory indicator although the color change passes through an intermediate green stage. Titration to a deep blue color gave the true end point. The Schmall extractor lends itself readily to nonaqueous analysis, since it gives essentially the same results as the Squibb separator, while its semiautomatic operation enhances its value to the analyst.

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Determination of the Purity of Digitalis Glycosides*

By JAMES E. MURPHY

A procedure is given for determining the amount of impurities present in digitalis glycosides and some of their preparations based on the fluorescence produced with a solution of propylene glycol and hydrochloric acid.

UNTIL THE LAST FEW YEARS there have been few methods available for checking the purity of the commercially available digitalis glycosides aside from the physical constants of the individual glycosides. In most cases these constants had been determined on samples which were themselves not pure and they leave much to be desired in their use for detecting the presence of other glycosides with very similar properties. Melting points, specific rotations, and the quantitative colorimetric reactions used have little value unless they can be supported by sensitive and specific tests for likely impurities. The use of paper chromatography has considerably aided in this task. However, as a rule, methods based on paper chromatography are only semiquantitative.

Members of the gitoxin series of the digitalis glycosides are the principal impurities in the official digitalis glycosides, digoxin, digitoxin, and lanatoside C, because of their tendency to form mixed crystals with the desired glycosides, which makes it very difficult to separate them by ordinary chemical means. The ring tests based on the Keller-Kiliani reactions (1, 2) have been used to detect these compounds in large amounts but they are not sensitive to better than 5–10 per cent even when carefully controlled. The sulfuric acid assay of McChesney, *et al.* (3),

* Received May 1, 1954, from the Analytical Research Laboratory, Burroughs Wellcome & Co., Inc. Tuckahoe, N. Y.

can determine relatively large amounts of gitoxin in both digoxin and digitoxin but it is not satisfactory for determining small amounts. The procedures of Banes and Carol (4) and Banes (5) for the analysis of digitoxin and digoxin preparations are more specific and accurate but are not suitable for routine control. The fluorescence reactions of Bellet (6), Pesez (7), Petit, *et al.* (8), and Jensen (9), are much more suitable for this problem and the method of Jensen, with some changes, has been used in our laboratory for the last few years with considerable success.

EXPERIMENTAL

Reagent.—Equal volumes of propylene glycol U. S. P., and hydrochloric acid were mixed and stored in a refrigerator. The solution was warmed to about 20° before using.

Standard Gitoxin Solution.—Ten milligrams of gitoxin was transferred to a 100-ml. volumetric flask and dissolved in enough chloroform-methanol 2:1 to bring to volume; 5 ml. of this solution was diluted to 50 ml. with the same solvent (1 ml. = 0.01 mg. gitoxin).

Procedure.—Ten milligrams of the digitalis glycoside was dissolved and diluted to 100 ml. in a volumetric flask with chloroform-methanol 2:1. A 1-ml. aliquot of this solution as well as 0.5- and 1-ml. aliquots of the standard gitoxin solution were transferred to 30-ml. beakers and evaporated just to dryness on a steam bath. Ten milliliters of the reagent was added to each of the cooled beakers with a fast delivery pipet, the solutions were stirred well with small glass rods and placed in a water bath kept at 20° for thirty minutes. Solutions were then transferred to photofluorometer cuvettes.

The sensitivity of a Coleman Model 12 photofluorometer was set at about 45, using a quinine sulfate solution which contained 0.15 μg./ml. and the B_1 and PC-1 filters. The instrument was then set to zero with a reagent blank. The fluorescence of the sample and standard solutions were determined. The per cent fluorescing substances as gitoxin were determined by reference to a standard curve or at lower values by direct comparison with the standard. When known amounts of gitoxin were added to a sample of purified digoxin, a straight-line relationship was obtained for the concentration of gitoxin up to 8%, after which there was a sloping off due to nonlinearity of instrument response. Upon dilution, the higher concentrations also gave a straight-line relationship.

Injections were tested by evaporating an aliquot equivalent to 0.1 mg. of the digitalis glycoside to dryness in a 30-ml. beaker and proceeding as above.

Tablets were tested after a suitable procedure to extract the active ingredient had been used or in some cases by merely stirring the finely powdered tablet with a known volume of chloroform-methanol 2:1, allowing to settle, and pipetting an aliquot equivalent to 0.1 mg. of the digitalis glycoside to a 30-ml. beaker and proceeding as above. Most of the common tablet excipients and diluents do not interfere.

The results obtained with the above procedure on some samples of digitalis glycosides are given in Table I.

TABLE I.—RESULTS OF TESTING DIGITALIS GLYCOSIDES FOR IMPURITIES

Sample		Fluorescing Substances as Gitoxin, %	
1	Digoxin, U. S. P. Reference Standard	3.6	3.5
2	Digitoxin, U. S. P. Reference Standard (*F*)	4.8	5.1
3	Digitoxin, U. S. P. Reference Standard (*G*)	2.3	2.3
4	Lanatoside C, U. S. P. Reference Standard	7.6[a]	
5	Digoxin[b]	0.2	
6	Digoxin	4.8	
7	Digoxin	1.0	
8	Digoxin	3.8	
9	Digitoxin[b, c]	0.37	0.35
10	Digitoxin	8.0	
11	Digitoxin[c]	7.8	7.3
12	Digitoxin[c]	35.2	34.5
13	Digoxin injection, 0.5 mg./ml.	5.4	5.5[d]
14	Digitoxin injection, 0.2 mg./ml.	7.1[d]	
14	Digitoxin injection, 0.2 mg./ml.	5.0[d]	

[a] Equivalent to 9.5% Lanatoside B.
[b] Specially purified samples of digoxin and digitoxin.
[c] Samples of digitoxin obtained from Dr. Banes. Sample 12 is the same as sample 12 assayed by Banes and Carol (4) which gave 37.2% gitoxin by their assay. All other samples were commercially available.
[d] These figures are per cent of the labeled amount of glycoside in the injection.

DISCUSSION

In the original procedure of Jensen (9), glycerol was used in the reagent but it was found that most samples of glycerol gave high reagent blanks especially on standing. Propylene glycol was found to be much more satisfactory. If the reagent is kept in the refrigerator, low blank values are obtained even with old reagent.

Digoxin, digitoxin, or lanatoside C, freed of impurities, give no response in this assay unless the assay is run at higher temperatures than 25°. According to Bellet (6), only glycosides having a hydroxyl group at the 16-carbon position respond. The reaction is not affected by the presence of digitoxose, glucose, or acetyl groups and, therefore, equimolar concentrations of gitoxigenin, gitoxin, *purpurea* glycoside B and lanatoside B give the same response.

In addition to the above members of the gitoxin series there are at least two other unknown glycosides which give a response. Unknown 'A', an impurity sometimes found in small amounts in digoxin gives a response approximately equal to that given by gitoxin. Unknown 'B', which can be found in both *D. lanata* and *D. purpurea* glycosides gives a response equal to about 120% that of gitoxin.

SUMMARY

1. A satisfactory method which is simple, sensitive, and reproducible is given for determining the total concentration of steroids of the gitoxin series in the official digitalis glycosides.

2. Two unknown glycosides also give a response in this assay and are estimated as gitoxin.

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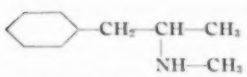
Cycloheptylalkylamines*

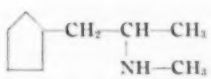
By WALTER C. McCARTHY and TERENCE H. BROWN†

A series of cycloheptylalkylamines has been prepared, starting with cycloheptanone. These compounds are structurally related to the commercially available sympathomimetic drugs, Benzedrex®, and Clopane®. β-Cycloheptylethylamine has been obtained by the catalytic hydrogenation of cycloheptenylacetonitrile. N-Methyl-β-cycloheptylethylamine has been produced by the reduction of N-methylcycloheptylacetamide with lithium aluminum hydride. β-Cycloheptylisopropylamine and N-methyl-β-cycloheptylisopropylamine have been synthesized by the catalytic reduction of cycloheptylacetone in the presence of ammonia and methylamine, respectively.

INTEREST in cycloalkylalkylamines has been heightened by the recent commercial availability of two such compounds, propylhexedrine (Benzedrex®) (I) containing a six-membered ring, and cyclopentamine (Clopane®) (II) containing a five-membered ring.

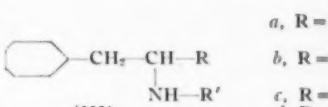


Propylhexedrine (I)



Cyclopentamine (II)

To obtain more information correlating cycloaliphatic ring size with pharmacological activity, we have undertaken the synthesis of suitable structural analogs containing larger and smaller ring systems. We are reporting herewith the preparation of four compounds containing the seven-membered ring and the side chains usually most active: the ethylamine (III*a*), N-methylethylamine (III*b*), isopropylamine (III*c*), and N-methylisopropylamine (III*d*) groups.



(III)

a, R = R′ = H
b, R = H, R′ = CH_3
c, R = CH_3, R′ = H
d, R = R′ = CH_3

Cycloheptanone was condensed with cyanoacetic acid, with ammonium acetate as the catalyst, to produce cycloheptylidenecyanoacetic acid. This was thermally decarboxylated, undoubtedly with concomitant rearrangement of the double bond which has been shown to occur with the six-membered ring (I), to produce cycloheptenylacetonitrile. This compound was reduced by hydrogen and Raney nickel, in the presence of ammonia, to β-cycloheptylethylamine (III*a*). Cycloheptenylacetonitrile was hydrolyzed to the corresponding acid, which was hydrogenated to cycloheptylacetic acid. This was converted through its acid chloride to N-methylcycloheptylacetamide, which, on reduction with lithium aluminum hydride, gave N-methyl-β-cycloheptylethylamine (III*b*). Cycloheptylacetyl chloride was condensed with the ethoxymagnesium derivative of malonic ester to produce the corresponding keto-ester, which on acid hydrolysis, smoothly decarboxylated to cycloheptylacetone. This ketone, on reductive amination under appropriate conditions, gave, respectively, β-cycloheptylisopropylamine (III*c*) and N-methyl-β-cycloheptylisopropylamine (III*d*).

The hydrochlorides of the four amines have been submitted to the Department of Pharmacology of the University of Washington for appro-

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priate testing, and their pharmacological evaluation will be published elsewhere.

EXPERIMENTAL[1]

Cycloheptanone.—This was prepared by ring enlargement, following a modification (2) of the method of Dauben and co-workers (3).

Cycloheptenylacetonitrile.—The method used was similar to that of Cope and co-workers (4) for the corresponding compound with the six-membered ring. From 123.4 Gm. (1.1 moles) of cycloheptanone and 85 Gm. (1.0 mole) of cyanoacetic acid with 3.0 Gm. (0.04 mole) of ammonium acetate catalyst, there was obtained 97 Gm. (72%) of product boiling at 110–120° (13–15 mm.). The analytical sample distilled at 121–122° (23 mm.); $n_D^{22.5}$ 1.4821, d_{25}^{25} 0.949.

Anal.—Calcd. for $C_9H_{13}N$: N, 10.36. Found: N, 10.36.

β-Cycloheptylethylamine, III a.—A solution of 20 Gm. (0.15 mole) of cycloheptenylacetonitrile in 200 ml. of absolute alcohol was saturated with dry ammonia. One teaspoonful of Raney nickel catalyst (5) was added and the hydrogenation was carried out at room temperature at 50 lb./in.² of hydrogen for twenty-four hours. The catalyst was filtered off, and the filtrate was distilled at reduced pressure. The fraction boiling at 95–100° (20 mm.) was collected, yield, 15.6 Gm. (74%). Constants were determined on a sample boiling at 103–104° (24 mm.): n_D^{25} 1.4838, d_{25}^{25} 0.996.

The *hydrochloride* was obtained from 8 Gm. of the amine dissolved in 50 ml. of absolute alcohol by saturation with dry hydrogen chloride gas and precipitation with dry ether. After recrystallization from an absolute alcohol-ether mixture, there was obtained 7.5 Gm. (73%) of the salt melting at 224–225° (dec.).

Anal.—Calcd. for $C_9H_{20}NCl$: N, 7.88. Found: N, 7.72.

The *phenylthiourea derivative* was recrystallized from dilute alcohol, m. p. 101.5–102°.

Anal.—Calcd. for $C_{16}H_{24}N_2S$: C, 69.51; H, 8.75. Found: C, 69.80; H, 8.47.

Cycloheptenylacetic Acid.—A mixture of 100 Gm. (0.74 mole) of cycloheptenylacetonitrile, 30 ml. of ethanol, 80 Gm. (2.0 moles) of sodium hydroxide, and 250 ml. of water was placed in a metal flask and refluxed for three days. After acidification with hydrochloric acid, the oily layer which separated was removed and the aqueous solution was extracted with three 50-ml. portions of ether. The ether extracts were mixed with the oily layer and dried over anhydrous sodium sulfate. The ether was removed by distillation and the residue was distilled under reduced pressure to yield 85 Gm. (75%) of product collected at 155–160° (15 mm.). A sample was further purified for analysis, b. p. 152–153° (16 mm.), n_D^{25} 1.4888, d_{25}^{25} 1.038.

Anal.—Calcd. for $C_9H_{14}O_2$: C, 70.10; H, 9.16. Found: C, 69.94; H, 9.17.

Cycloheptylacetic Acid.—A teaspoonful of Raney nickel catalyst (5) was added to a solution of 70 Gm. (0.46 mole) of cycloheptenylacetic acid in 200 ml. of absolute alcohol, and the mixture was hydrogenated at 40° at 50 lb./in.² pressure for eighteen hours. The Raney nickel was removed by filtration, the solution was made alkaline with aqueous sodium hydroxide, and the alcohol was distilled off. After adding 100 ml. of water to the residue, the mixture was acidified with hydrochloric acid, and then extracted with three 100-ml. portions of ether. The ether extracts were dried over anhydrous sodium sulfate, the ether was removed, and the residue was distilled under reduced pressure. A yield of 62 Gm. (87%) was obtained in the fraction boiling at 145–150° (12 mm.). The analytical sample distilled at 159–161° (15 mm.); n_D^{25} 1.4743, d_{25}^{25} 1.023.

Anal.—Calcd. for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 68.97; H, 10.21.

Cycloheptylacetyl Chloride.—A mixture of 40 Gm. (0.26 mole) of cycloheptylacetic acid and 59 Gm. (0.5 mole) of thionyl chloride was reacted at room temperature for about twenty minutes. The excess thionyl chloride was removed under reduced pressure; dry benzene was added and then removed under reduced pressure, with only gentle warming, to remove the last traces of thionyl chloride from the reaction mixture. (If the acid was refluxed with thionyl chloride, or if all the thionyl chloride was not removed before distillation was started, a tar was produced, and no acid chloride was obtained.) The residue was distilled under reduced pressure. A yield of 41.5 Gm. (93%) was obtained in the fraction which came over at 105–110° (15 mm.).

The *anilide derivative* was recrystallized from dilute alcohol, m. p. 82–83°.

Anal.—Calcd. for $C_{15}H_{21}NO$: C, 77.88; H, 9.15. Found: C, 77.98; H, 8.45.

N-Methylcycloheptylacetamide.—Liquid methylamine was prepared by dropping a solution of 13 Gm. (0.2 mole) of methylamine hydrochloride in 100 ml. of water onto sodium hydroxide pellets, passing the gas through a drying tower filled with calcium oxide, and condensing it in a flask immersed in a dry-ice bath. Cycloheptylacetyl chloride, 7 Gm. (0.04 mole), was added dropwise to the excess liquid methylamine still immersed in the dry-ice bath. After the addition was complete, the mixture was allowed to warm up to room temperature. Water (100 ml.) was added to the residue, and this mixture was extracted with three 50-ml. portions of ether. The ether extracts were dried over anhydrous sodium sulfate, the ether was removed under reduced pressure, and the remaining product was vacuum distilled. The fraction which came over at 180–185° (15 mm.), 3.1 Gm. (50%), subsequently crystallized. After recrystallization from *n*-hexane, it melted at 53–54°.

Anal.—Calcd. for $C_{10}H_{19}NO$: N, 8.27. Found: N, 8.36.

N-Methyl-β-cycloheptylethylamine, III b.—A solution of 3.1 Gm. (0.02 mole) of N-methylcycloheptylacetamide in 20 ml. of dry ether was added dropwise, with constant stirring, to a solution of 0.86 Gm. (0.023 mole, 50% excess) lithium aluminum hydride (6) in ether. After all the amide was added, the mixture was refluxed for one hour. It was then hydrolyzed with water, acidified with hydrochloric acid and extracted with three 25-ml. portions of ether. The aqueous layer was made alkaline with sodium hydroxide and extracted with four 50-ml. portions of ether. Because of the gelatinous precipitate, a centrifuge was used to sepa-

[1] All melting points are uncorrected.

rate the layers. The ether extracts were washed with water, and dried over anhydrous sodium sulfate. The ether was removed under reduced pressure, and the remaining material was vacuum distilled. A yield of 1.3 Gm. (46%) was obtained in the fraction boiling at 100–105° (20 mm.); constants were determined on a sample boiling at 104–105° (17 mm.): $n_D^{24.5}$ 1.4686, d_{25}^{25} 0.873.

The *hydrochloride* was obtained from 5 Gm. of the amine in 78% yield, m. p. 178–179°, by the method used on β-cycloheptylethylamine.

Anal.—Calcd. for $C_{10}H_{22}NCl$: N, 7.31. Found: N, 7.10.

The *phenylthiourea derivative* was recrystallized from dilute alcohol, m. p. 112.5–113°.

Anal.—Calcd. for $C_{17}H_{26}N_2S$: C, 70.29; H, 9.02. Found: C, 70.23; H, 8.63.

Cycloheptylacetone.—The general method of Walker and Hauser (7) for the synthesis of methyl ketones was used. From 5.35 Gm. (0.22 mole) of magnesium turnings, 35.2 Gm. (0.22 mole) of diethyl malonate, and 34.8 Gm. (0.20 mole) of cycloheptylacetyl chloride, there was obtained 25.6 Gm. (83%) of cycloheptylacetone in the fraction boiling at 95–100° (12–13 mm.). The analytical sample distilled at 98–99° (14 mm.); n_D^{29} 1.4628, d_{25}^{25} 0.960.

Anal.—Calcd. for $C_{10}H_{18}O$: C, 77.87; H, 11.76. Found: C, 77.73; H, 12.03.

The *2,4-dinitrophenylhydrazone derivative* was recrystallized from dilute alcohol, m. p. 124.5–125°.

Anal.—Calcd. for $C_{16}H_{22}O_4N_4$: C, 57.47; H, 6.63. Found: C, 57.51; H, 6.65.

β-Cycloheptylisopropylamine, III c.—Ten grams (0.065 mole) of cycloheptylacetone was added to a solution of 6 Gm. (0.35 mole) of dry ammonia in 100 ml. of absolute alcohol. The mixture was hydrogenated for twenty-four hours over Raney nickel catalyst (5) at room temperature and 50 lb./in.² pressure. The catalyst was filtered off and the alcohol was removed by distillation. The residue was dissolved in 5% hydrochloric acid and extracted with ether. The aqueous layer was then made alkaline with 10% sodium hydroxide solution and extracted with four 90-ml. portions of ether. This ether extract was washed with water, dried over anhydrous sodium sulfate and the ether was distilled off. Upon distillation of the residue under reduced pressure, there was obtained 4.4 Gm. (56%) of the amine in the fraction collected at 105–110° (20 mm.). Constants were determined on a sample collected at 101–102° (18 mm.): n_D^{25} 1.4686, d_{25}^{25} 0.880.

The *hydrochloride* was obtained from 10 Gm. of the amine in 78% yield, m. p. 210–211°, by the method used on β-cycloheptylethylamine.

Anal.—Calcd. for $C_{10}H_{22}NCl$: N, 7.31. Found: N, 7.30.

N-Methyl-β-cycloheptylisopropylamine, III d.—This preparation followed the method used for III*c*. From 10 Gm. (0.065 mole) of cycloheptylacetone and 6 Gm. (0.19 mole) of methylamine, there was obtained a yield of 7.1 Gm. (65%) of the secondary amine collected at 110–120° (20 mm.). Constants were determined on a sample which distilled at 111–112° (19 mm.): n_D^{29} 1.4639, d_{25}^{25} 0.764.

The *hydrochloride* was obtained from 7.1 Gm. of the amine in 62% yield, m. p. 135–136°, by the method on β-cycloheptylethylamine.

Anal.—Calcd. for $C_{11}H_{24}NCl$: C, 64.20; H, 11.76. Found: C, 64.49; H, 11.98.

The *phenylthiourea derivative* was recrystallized from dilute alcohol, m. p. 94.5–95°.

Anal.—Calcd. for $C_{18}H_{28}N_2S$: N, 9.20. Found: N, 9.60.

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CORRECTION

In the article entitled "A Method of Evaluating Enteric Coatings Using the White Rat," by F. C. Hammerness and C. H. Waldon [THIS JOURNAL, **43**, 357(June 1954)] a footnote appears on page 358 which reads as follows: "Pyridium®, a registered trademark of Merck and Company, Inc." This footnote should have read, "Pyridium®, a registered trademark of the Nepera Chemical Company."

Dietary Feeding of a New Methylcellulose Preparation to Rats*

By D. D. McCOLLISTER and F. OYEN

Methocel® 2602, a new methylcellulose preparation, is intermediate in gel point properties to Methocel® and Methocel® HG, and is superior to both in compatibility with organic solvents. The results of the feeding of Methocel® 2602 in the diet of rats for a period of one hundred and twenty-one days at levels of 1, 3, 10, and 30 per cent indicate that this new methylcellulose preparation is extremely low in chronic oral toxicity. Thus, it is believed that Methocel® 2602 is safe for uses in the drug, food, and pharmaceutical fields similar to those in which Methocel®, and Methocel® HG have been found suitable.

METHOCEL®[1] 2602 is a hydroxypropyl methyl cellulose similar to Methocel® in most properties except gel point and compatibility with organic solvents. It is intermediate in gel point properties to Methocel® and Methocel® HG, and is superior to both of the older products in compatibility with organic solvents. These methylcellulose preparations are chemically inert materials of high molecular weight. In each case, the methoxyl group substitution amounts to 29–30 per cent. Methocel® HG contains 2–4 per cent hydroxypropoxyl groups; whereas, Methocel® 2602 consists of 6–7 per cent hydroxypropoxyl substitution.

A short-term chronic dietary feeding of Methocel® 2602 to rats was conducted in order to obtain data for comparison with the information(1–3) available on Methocel® and Methocel® HG.

EXPERIMENTAL PROCEDURE

The sample of Methocel® 2602 used in these studies was of plant production grade. It was a white powder with a melting point of 140–160°. The diets to be fed to the experimental animals were prepared by the thorough mixing of the proper amount of Methocel® 2602 with the basic rat diet to make compositions containing 30, 10, 3, and 1% Methocel® 2602. The ingredients of the control and basic ration used in this investigation were as follows: whole wheat, freshly ground, 55% by weight; dried whole milk, 25% by weight; dried meat residue, 12% by weight; dried yeast (Red Star), 5% by weight; iodized table salt, 2% by weight; and calcium carbonate, 1% by weight.

All of the rats used in this work were progeny of stock obtained by this laboratory from the Wistar Institute in 1938. These animals were maintained on a stock diet from the time of weaning until they were from two to three months of age, when they were divided according to body weight into well-matched groups of 10 male and 10 female rats each and started on the diets containing 0.0 (control), 30, 10, 3, and 1% Methocel® 2602.

Five rats were caged together in wire-bottom cages and were allowed free access to food and water at all times. All of the rats were weighed twice each week throughout the experimental period. Records were kept of body weights, general appearance, mortality, and average daily food consumption. Whenever possible, failing animals were sacrificed when moribund in an effort to ascertain the cause of impending death. Terminal hematological values were obtained from a group of 5 female control rats and 5 female rats that received the 10% dietary level of Methocel® 2602. After 121 days on the experimental diets, all of the surviving rats were starved overnight, weighed, killed by decapitation and examined. The livers and kidneys from each rat were weighed. Hematoxylin- and eosin-stained sections from these organs, as well as from the pancreas, lung, heart, spleen, testes, and adrenals, were prepared for histologic examination.

EXPERIMENTAL RESULTS

The dietary level of 30% Methocel® 2602 obviously was not well tolerated by the male and female rats that received it. A great deal of food was wasted. There was a marked retardation of growth (Fig. 1). The rats became rough and unkempt in appearance. In many instances, the fur came out in large patches and the feces became soft and sticky. Four male rats and six female rats failed to survive the full 121-day period. The symptoms in each instance were those attributed to undernourishment, approaching starvation. At autopsy of the six male and four female rats that survived the full experimental period, there was no evident gross pathology. Organ weight studies (Table I) revealed no effects inconsistent with the response expected from animals in such an obviously undernourished state. There was no evidence of adverse effects as a result of a microscopic examination of any of the tissues.

In regard to the groups of rats receiving the dietary level containing 10% Methocel® 2602, there was a slight retardation of growth for the male rats (Fig. 1). This effect was not produced in the group of female rats on this dietary level. There was no evidence of adverse effects in the male and

* Received March 4, 1954, from the Biochemical Research Department, The Dow Chemical Company, Midland, Mich.

[1] Methocel® is a registered trademark of The Dow Chemical Company.

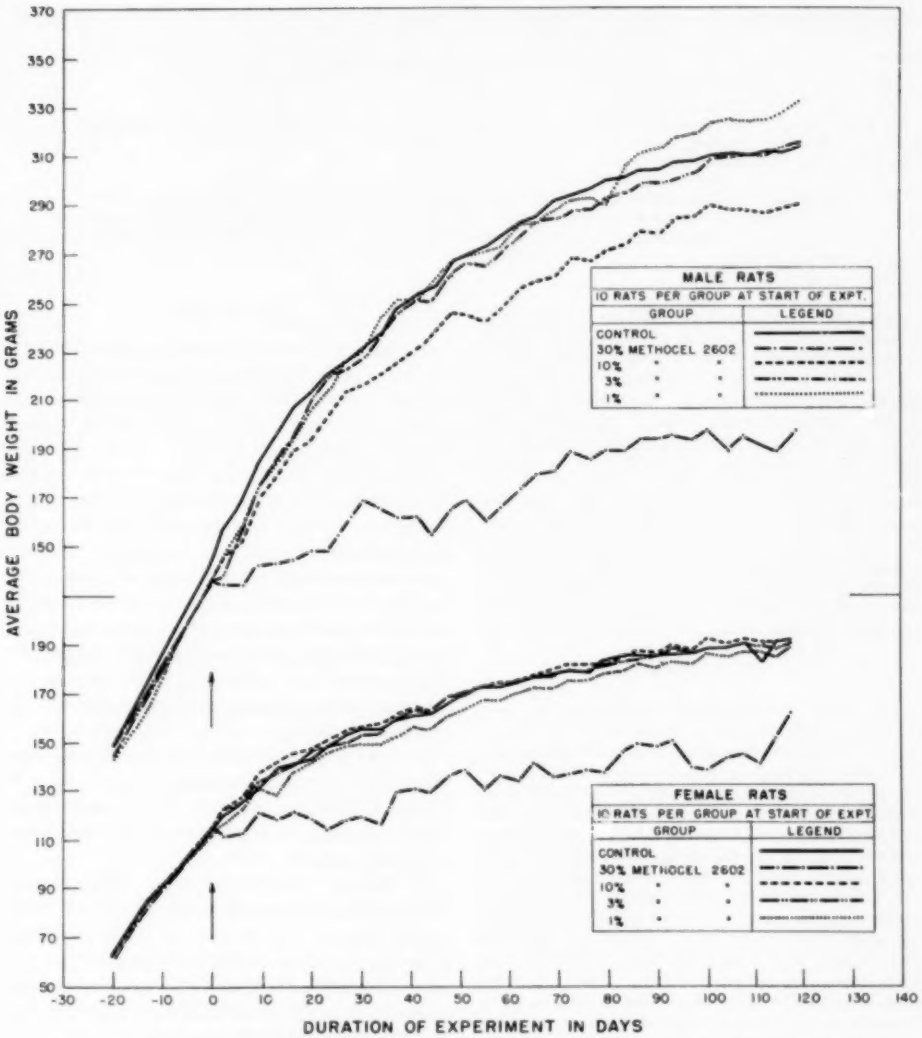

Fig. 1.—Average body weight curves for rats that received diets containing Methocel® 2602.

TABLE I.—AVERAGE FINAL BODY AND ORGAN WEIGHTS FROM MALE AND FEMALE RATS THAT RECEIVED VARIOUS CONCENTRATIONS OF METHOCEL® 2602 IN THE DIET FOR A PERIOD OF 121 DAYS

Per Cent in Diet	Sex	No. of Rats	Body Wt. (Gm.)	Liver		Kidney	
				Gm.	Gm./100 Gm.	Gm.	Gm./100 Gm.
Control	M	7	303	6.71	2.21	1.82	0.60
30	M	6	174	4.58	2.64	1.38	0.80
10	M	9	278	6.35	2.27	1.76	0.63
3	M	8	305	6.67	2.19	1.89	0.62
1	M	9	312	7.27	2.24	1.94	0.60
Control	F	9	185	4.79	2.59	1.29	0.70
30	F	4	170	4.25	2.50	1.20	0.71
10	F	8	179	4.49	2.50	1.25	0.70
3	F	10	185	4.61	2.48	1.27	0.68
1	F	9	180	4.58	2.53	1.23	0.69

TABLE II.—AVERAGE HEMATOLOGICAL VALUES FROM FEMALE RATS THAT RECEIVED A DIET CONTAINING 10% METHOCEL® 2602 FOR A PERIOD OF 121 DAYS

Determination	Average Value Per Group of 5 Rats	
	Control	10% Methocel® 2602
Total erythrocytes	8.65×10^6	8.98×10^6
Total leucocytes	12.8×10^3	13.4×10^3
Hemoglobin	14.0 g./100 cc.	14.1 g./100 cc.
Differential count		
Neutrophiles	20.8%	21.8%
Lymphocytes	76.8%	77.4%
Monocytes	0.2%	0.4%
Eosinophiles	2.2%	0.4%

female rats that received the dietary level of 10% Methocel® 2602 for a period of 121 days as judged by general appearance and behavior, food consumption, mortality, gross examination at autopsy, organ weights, or micropathologic examination of the tissues. Terminal hematological studies on five of the female rats gave only normal values, closely comparable with those obtained from a similar group of control rats (Table II).

The groups of male and female rats that received diets containing 3% and 1% Methocel® 2602 for a period of 121 days showed no evidence whatsoever of adverse effects as judged by growth, mortality, general appearance, and behavior, final body and organ weights, and gross and microscopic examination of the tissues.

DISCUSSION

The results of these dietary feeding studies show that Methocel® 2602 is extremely low in chronic oral toxicity. In this respect, it appears to be similar to regular Methocel® (1) and Methocel® HG (2, 3). Hodge, *et al.* (2), fed Methocel® HG in the diets of rats for a period of two years at levels of 1, 5, and 20%. Growth retardation, exhibited by the male rats maintained on the 20% diet, was the only effect attributed to the experimental material.

Regular Methocel® and Methocel® HG have been widely used in the pharmaceutical field as thickening agents and as protective colloids in emulsions and dispersions. Methocel® 2602, because of its particular range of gelation temperatures and degree of solubility and compatibility with organic solvents, is suitable for many applications requiring improved performance. It has been estimated that the total Methocel® 2602 content of liquid emulsions or of aqueous dispersions of solid pharmaceutical material would run from 1–5% by weight. Due to the close similarity in chemical composition to Methocel® and Methocel® HG and the comparable low order of chronic oral toxicity, it is believed that Methocel® 2602 is safe for such uses.

SUMMARY

1. Methocel® 2602 was fed in the diet of rats (10 males and 10 females per group) for a period of one hundred and twenty-one days at levels of 30, 10, 3, and 1 per cent. There was a marked retardation of growth in the group of male and female rats that received the 30 per cent dietary level and mortality was high. There was a slight retardation of growth in the group of male rats receiving the 10 per cent dietary level but no ill-effects by any criterion in the female rats. Under-nutrition, due to non-nutritive "bulk" in the diet, is indicated as responsible for the effects on growth. No specific tissue action was revealed in the organ weight and histopathologic studies even at the 30 per cent concentration.

2. The groups of male and female rats that received diets containing 3 per cent and 1 per cent Methocel® 2602 for a period of one hundred and twenty-one days showed no evidence whatsoever of adverse effects.

3. It it concluded that, like regular Methocel® and Methocel® HG, Methocel® 2602 is extremely low in chronic oral toxicity. Thus, it is believed that Methocel® 2602 is safe for uses in the drug, food, and pharmaceutical fields similar to those in which Methocel® and Methocel® HG have been found suitable.

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Species Differences in the Excretion and Distribution of *p*-Chloroacetanilide-Cl^{36}*

By JOSEPHINE M. GRYDER, MARY F. ARGUS, MARJORIE P. NEWELL, and F. E. RAY†

p-Chloroacetanilide-Cl^{36} was synthesized and its distribution and excretion studied in rats and guinea pigs. The results obtained indicate a species difference, elimination of the compound being slower in the rat. The toxicity of the solvent used, propylene glycol, is pointed out.

ACETYL DERIVATIVES of aniline have found wide usage as drugs since the discovery, in 1886, that aniline and acetanilide have powerful antipyretic and analgesic properties. Studies carried out on the metabolism of these compounds show that the main product found in the urine as a result of the metabolism of aniline is *p*-aminophenol, although a small amount of *o*-aminophenol has also been found. These compounds occur in the urine not only in the free state, but also as the ethereal sulfate and glucuronide.

There has been, in the past, a belief that aniline and acetanilide are interconvertible *in vivo*, but this is not wholly true. It has been shown that the extent of deacetylation of the aromatic acetylamino group depends on the animal species, deacetylation being extensive in the cat, dog, and pigeon, but very small in man and the rabbit. Since it has been shown that dog liver contains an aromatic deacylase which rapidly deacetylates acetanilide, while rabbit liver has little effect, it follows that aniline and acetanilide are likely to produce similar metabolites in animals such as the dog and cat, and different ones in man and the rabbit (1).

In the rabbit, acetanilide is converted mainly into the ethereal sulfate and glucuronide of *p*-acetylaminophenol, no more than 6–7 per cent of a dose being excreted as compounds containing free aromatic amino groups. This *p*-acetylaminophenol is responsible for the therapeutic activity of acetanilide, while its toxic effects are caused by the aniline produced. This toxicity is lowered in the case of phenacetin (acetophenetidin or *p*-ethoxyacetanilide). *p*-Acetylaminophenol is also the major metabolite of phenacetin, but here it is produced by de-ethylation rather than oxidation *in vivo*, as is the case with acetanilide. The extent of deacetylation of phenacetin is less than that of acetanilide, and this fact seems to be correlated with its lesser toxicity.

The present study was undertaken to determine the effect on acetanilide metabolism when the vulnerable *p*-position was blocked with chlorine-36, and also to determine whether a species difference exists between the rat and the guinea pig. Since it is possible that the extent of the activity of a compound may depend on the degree to which it is metabolized in the animal body, this study may serve to elucidate some of the physiological properties of aromatic amines.

EXPERIMENTAL

Preparation of p-Chloroacetanilide-Cl^{36}.—This procedure was designed to utilize best the Cl^{36}. The radioactive chlorine was obtained as very dilute (0.215 *N*) hydrochloric acid. A volume of 26.7 ml. of this HCl^{36} (containing 100 μc. of activity) was neutralized with 20% NaOH and evaporated until only the moist $NaCl^{36}$ remained in the tube. This tube was then used as a generator for Cl^{36} gas. Six normal H_2SO_4 (1.8 ml.; 0.01 mole) was added to convert the $NaCl^{36}$ to HCl^{36} and this was oxidized to Cl^{36} gas with $KMnO_4$ (1.58 Gm.; 0.01 mole) and 36 *N* H_2SO_4 (1.1 ml.; 0.02 mole). This gas was passed into acetanilide (1.35 Gm.; 0.01 mole) dissolved in 10 ml. glacial acetic acid in an 80-ml. centrifuge tube. The chlorine-36 generator was then swept out with base chlorine gas from a second generator where 2.21 Gm. of $KMnO_4$ (0.014 mole) and 1.1 ml. 36 *N* H_2SO_4 (0.02 mole) were used to oxidize 1.68 ml. of 8 *N* HCl (0.014 mole). The entire system was finally swept out with CO_2. The *p*-chloroacetanilide-Cl^{36} was brought down by neutralizing the acetic acid solution with 35 ml. of 20% NaOH. The product was washed three times with water; redissolved in 6 ml. glacial acetic acid; reprecipitated with 20 ml. of 20% NaOH; again washed three times with water and finally extracted with 6 ml. warm chloroform. All these purification steps were carried out in the same centrifuge tube, using a filter stick to remove filtrates and washing fluids. The final product, melting at 175–177°, was obtained in a yield of 0.64 Gm. and had a specific activity of 130,000 d. p. m./mg. Values ranging from 174–179° are given in the literature (5–7) for the pure compound.

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The hydrochloric acid-Cl^{36} was obtained from the Oak Ridge National Laboratory on allotment by the Atomic Energy Commission.
† With the technical assistance of Charles J. Osterholt, Jr.

Distribution of *p*-Chloroacetanilide-Cl^{36}.—Five male stock guinea pigs and six Sprague-Dawley rats were used. All animals were injected intraperitoneally with 100 mg. *p*-chloroacetanilide-Cl^{36} in 2–3 ml. propylene glycol/Kg. of body weight. Preliminary experiments had shown that this dose was tolerated by the animals. After injection, the animals were placed in metabolism cages which permitted the separate collection of urine and feces.

Urine and feces from one rat and one guinea pig were collected every twelve hours as long as any radioactivity was present. Distribution studies were carried out at the time of maximum excretion and at a time halfway between the starting point and the time of maximum excretion.

At the appointed time, the other animals were anesthetized with ether and a sample of blood collected by heart puncture. The organs were removed, washed, blotted on filter paper, and weighed. The liver, spleen, kidneys, stomach, small intestine, colon, and cecum were worked up separately. The heart, lungs, adrenals, gonads, brain, thymus, pancreas, esophagus, and bladder were combined in one fraction designated as "miscellaneous." The feces and contents of the gastrointestinal tract (stomach, small intestine, cecum, and colon) were removed and dried for twenty-four hours at 60°. Following removal of the organs, the carcass (skin, bones, muscles, and subcutaneous tissue) was autoclaved at 15 lb. pressure for twenty-five minutes. The organs, miscellaneous fraction, and autoclaved carcass were homogenized separately in a Waring Blendor in the presence of 25 ml. of 1% sodium hydroxide per 10 Gm. of tissue. Five drops of the wetting agent Tergitol®/10 Gm. tissue, and a few drops of capryl alcohol to prevent foaming was added. The suspension was allowed to stand for twenty-four hours at 5°. The mixture was agitated again in the Waring Blendor. A 1-ml. portion of each sample was then placed on a nickel-plated planchet and dried.

The dried feces and gastrointestinal contents were ground in a mortar to a fine powder and mixed in a Waring Blendor as above, except that 50 ml. of 1% sodium hydroxide/10 Gm. of sample were used. The planchets were prepared in the same manner. One-milliliter samples of blood cells, blood plasma, and urine were plated directly on planchets. Radioactivity measurements were made in an internal-type counter (Q-Gas Chamber and Nuclear Instrument and Chemical Corporation Scaler, Unit Model 162 with an efficiency of 40%). Each sample was counted for a twenty-minute period and the net counts above background recorded. The concentration of *p*-chloroacetanilide-Cl^{36} in the samples was determined by reference to a previously established calibration curve. This curve was made from planchets prepared in the same manner as the experimental samples and containing known amounts of *p*-chloroacetanilide-Cl^{36}. Feces standards were used for determining the feces and gastrointestinal contents; liver standards for the organs, miscellaneous fraction, carcass and blood cells; and urine standards for the urine and blood plasma. Because of the long half-life of chlorine-36 (4.4×10^5 years), no correction for its decay was necessary.

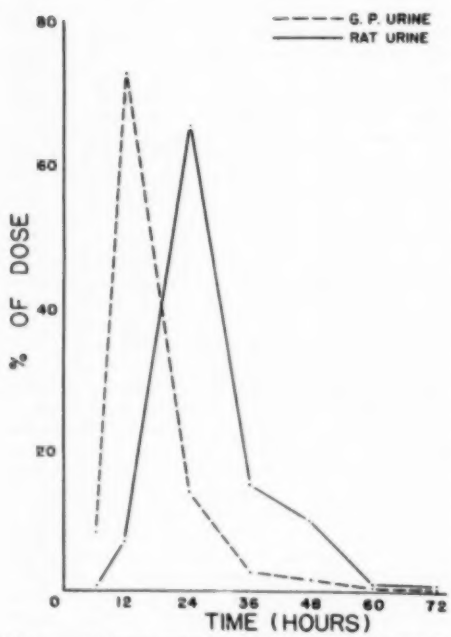

Fig. 1.—Urinary excretion of 100 mg. *p*-chloroacetanilide-Cl^{36}/Kg. by the rat and the guinea pig.

RESULTS AND DISCUSSION

The excretion of radioactivity in the urine and feces is given in Fig. 1. It will be observed that elimination is much more rapid in the guinea pig than in the rat. At six hours, 0.28% of the dose was found in the rat urine compared with 7.4% in the guinea pig, while at twelve hours the values were 9.3 and 75.7%. At twenty-four hours, as much of the dose was found in the rat urine as was found in the guinea pig urine at twelve hours. The rat required eighty-four hours to eliminate completely the administered dose, as compared to seventy-two hours in the guinea pig. Only small amounts were eliminated in the feces, but more was eliminated in the rat's feces. The results of the distribution studies are given in Table I.

Although both species had appreciable amounts of radioactivity in both blood cell and plasma, the rat had considerably more, especially at twelve hours. This might indicate that a more permanent bond was formed between blood and compound, or that the rat blood receives and carries more of the compound. Since the liver, spleen, and kidneys of the rat also contain a higher percentage of radioactivity, the latter theory seems more likely. At six hours the ratio of compound in the liver of rat to guinea pig was 2:1, while at twelve hours this had increased to 3:1. Since the concentration in rat liver was unchanged, this change in ratio reflects the more rapid elimination by the guinea pig. It is possible that the compound is more profoundly changed during its slow passage in the rat. However, the metabolites excreted by the two species must be identified and compared before this is definitely known. This work is now underway.

TABLE I.—DISTRIBUTION OF RADIOACTIVITY IN RATS AND GUINEA PIGS 6, 12, AND 24 HOURS AFTER INTRAPERITONEAL INJECTION OF 100 MG. p-CHLOROACETANILIDE-Cl^{36} KG.

	6 Hours				12 Hours				24 Hours	
	Conc. μg./Gm. Tissue[c]		% of Dose		Conc. μg./Gm. Tissue[c]		% of Dose		Conc. μg./Gm. Tissue[c]	% of Dose
Tissue	Rat[a]	Guinea Pig	Rat[a]	Guinea Pig[a]	Rat	Guinea Pig[a]	Rat	Guinea Pig[a]	Rat[a]	Rat[a]
Urine	34.6	460.0	0.28	7.4	36.50	615.	9.3	75.5	855.0	63.2
Feces	1.5	50.5	0.01	0.22	[b]	[b]	[b]	[b]	107.0	0.25
Blood cells	23.6	23.2	0.78	0.68	40.5	6.0	1.4	0.18	24.2	0.80
Blood plasma	23.5	15.8	0.75	0.46	21.7	7.3	0.73	0.22	14.1	0.47
Liver	67.0	29.0	2.60	1.20	51.0	16.5	2.4	0.80	17.5	0.70
Spleen	14.8	14.5	0.04	0.04	30.8	3.0	0.10	0.01	6.2	0.01
Kidneys	78.0	29.5	0.80	0.28	60.0	29.5	0.60	0.28	12.0	0.10
Stomach	24.2	36.0	0.16	0.34	41.0	16.2	0.27	0.14	5.8	0.02
Small intestine	57.0	45.2	1.20	1.03	88.2	17.5	2.0	0.32	59.0	0.91
Cecum	29.0	17.5	0.14	0.18	20.0	14.5	0.13	0.50	20.5	0.06
Colon	40.5	34.0	0.27	0.48	28.0	17.0	0.15	0.17	12.5	0.05
Stomach content	98.5	70.5	0.12	1.20	37.0	50.0	0.32	0.93	45.0	0.02
Small intestine content	161.0	258.5	0.34	0.36	269.0	148.5	1.8	0.31	1375.	0.83
Cecum content	78.0	83.5	0.24	2.00	23.0	77.5	0.08	1.60	48.0	0.42
Colon content	29.0	81.0	0.04	0.06	52.0	28.0	0.09	0.08	61.5	0.04
Misc.	52.5	40.0	1.40	1.40	50.2	16.0	1.7	0.48	12.2	0.38
Carcass	131.5	101.1	86.20	77.0	192.8	17.8	78.5	13.4	48.5	26.20
Total			95.37	94.33			99.57	94.92		94.46

[a] Average values from 2 animals.
[b] No feces obtained at 12 hours.
[c] Or μg./ml. urine, blood cells, or blood plasma.

Although the compound was injected into the peritoneum, substantial amounts are found in the stomach contents of both animals, with somewhat more being present in the stomach of the guinea pig. The fact that the guinea pig has an all-glandular stomach, while the rat's stomach is divided into a glandular stomach and a forestomach possibly accounts for the higher percentage found in the guinea pig. Appreciable amounts were also found in the intestines of the two animals. The compound possibly found its way there through the bile, as well as from the stomach.

Not much difference is found in the miscellaneous fraction of the two species at six hours, but at twelve hours the miscellaneous fraction of the rat contained considerably more activity than that of the guinea pig. The same holds true for the carcass. The rat seems to have the ability to store the radioactive material. Even at twenty-four hours, the rat's blood contains more activity than was found in that of the guinea pig at either time interval. The small intestine of the rat at twenty-four hours still contained nearly three times as much activity as did that of the twelve-hour guinea pig. The small intestine contents of the rat contained considerably more activity than was found in the guinea pig at either time. It will be remembered that at twenty-four hours 93% of the activity had been eliminated by the guinea pig, whereas less than 70% had been eliminated by the rat. Previous work in this Laboratory has shown that *p*-chloroacetanilide, in the amount here used, produces far more methemoglobin in the rat than in the guinea pig (2). The present results serve to explain this difference.

The solvent used, propylene glycol, has been considered nontoxic and has been used as an inert solvent for administration of compounds both orally and parenterally. However, the same studies (2) have shown that the solvent is toxic to rats and guinea pigs. Moreover, it was found to reduce the formation of methemoglobin by *p*-chloroacetanilide in both species; again the effect was larger in the rat than in the guinea pig. It has also been shown in this laboratory that when aqueous toluidine blue O is injected intraperitoneally into rats, it is secreted by the stomach; when injected in propylene glycol, it is not (3). In the present study propylene glycol was used as a solvent for purposes of comparison with previous studies on aminofluorene (4).

SUMMARY

1. *p*-Chloroacetanilide-Cl^{36} was synthesized and the distribution was studied at 6 and 12 hours in the guinea pig and at 6, 12, and 24 hours in the rat after intraperitoneal injection.

2. It was found that the rat excretes the compound more slowly than does the guinea pig. The percentage of radioactive material was also considerably higher in liver, kidneys, blood plasma and blood cells of the rat.

3. Substantial amounts of radioactivity were found in the gastrointestinal tract of both species.

4. It was pointed out that the solvent used, propylene glycol, is toxic to both species, and changes the blood picture in animals injected with *p*-chloroacetanilide.

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The Synthesis of Some Lactones Related to the Cardiac Aglycones*

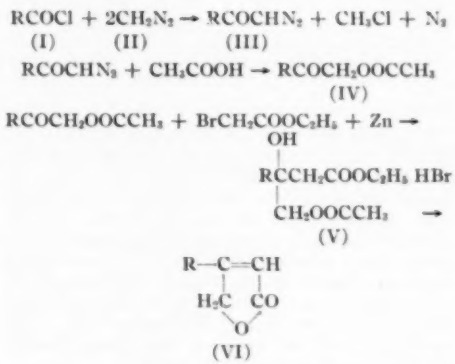
By JAMES W. CONINE† and JAMES W. JONES

Six lactones, β-(3,4-dimethoxyphenyl)-$\Delta^{\alpha,\beta}$ butenolide, β-(3-methoxy-4-hydroxyphenyl)-$\Delta^{\alpha,\beta}$ butenolide, β-hexahydrobenzyl-$\Delta^{\alpha,\beta}$-butenolide, β-(*p*-methoxybenzyl)-$\Delta^{\alpha,\beta}$ butenolide, β-(*o*-methoxybenzyl)-$\Delta^{\alpha,\beta}$ butenolide, and β-[γ-(*p*-methoxyphenyl)propyl]-$\Delta^{\alpha,\beta}$ butenolide were prepared. Seven acetoxymethyl ketones, 3-methoxy-ω-acetoxyacetophenone, 3-methoxy-ω,4-diacetoxyacetophenone, hexahydrobenzyl acetoxymethylketone, *p*-methoxybenzyl acetoxymethyl ketone, *o*-methoxybenzyl acetoxymethyl ketone, γ-(*p*-methoxyphenyl)propyl acetoxymethyl ketone, and 3,4-dimethoxy-ω-acetoxyacetophenone were prepared as intermediates for the lactone syntheses. High-pressure reduction of benzoic acid, phenylacetic acid, and *o*-methoxybenzoic acid in alkaline solution over W-4 Raney nickel was carried out. The ultraviolet spectra of the lactones and selected related compounds were determined.

SINCE the elucidation of the structures of the cardiac glycosides, a number of attempts have been made to synthesize the naturally occurring compounds or to prepare new compounds with comparable activity. Neither objective has been accomplished completely. Allouzarigenin, a representative aglycone of the inert allo-glycoside type, has been prepared by a lengthy series of reactions (1–3). Model compounds in which the aromatic ring is conjugated with the unsaturated lactone ring have been prepared (4–6) but these are inactive with the possible exception of β (β-naphthyl)-$\Delta^{\alpha,\beta}$-butenolide (7). $\Delta^{\alpha,\beta}$-Butenolides having a β-substituent of the "stilbestrol" and "hexestrol" types have also been prepared (8, 9). The methyl ester of the "stilbestrol" derivative was found to have a potency one-one-hundredth that of strophanthin (8).

In this work, a series of β-substituted $\Delta^{\alpha,\beta}$-butenolides having both aryl and aralkyl substituents was prepared for future pharmacological examination. In the aralkyl series, there is no longer the possibility of conjugation between the aromatic substituent and the lactone ring such as occurs in the β-aryl-$\Delta^{\alpha,\beta}$-butenolides. This results in marked differences in the physical properties and the ultraviolet spectra.

The method of preparation of the butenolides was essentially that used by Elderfield and his co-workers (4).



The acid chloride I, in which R was the substituent desired in the *beta*-position of the $\Delta^{\alpha,\beta}$-butenolide, was reacted with an excess of diazomethane II to form the corresponding diazoketone III. The diazoketone was treated with glacial acetic acid to form the acetoxymethyl ketone IV. Upon treatment with zinc and ethyl bromoacetate, the acetoxymethyl ketone was converted to a β-hydroxy-γ-acetoxy ester which was hydrolyzed and dehydrated to the *beta*-substituted $\Delta^{\alpha,\beta}$-butenolide VI.

EXPERIMENTAL

Reduction of Acids Containing an Aromatic Ring.—Three acids were subjected to reduction in aqueous alkaline solution by the method of Macbeth (10–12). Twenty-two hundredths of a mole of acid was dissolved in 105 ml. of 9% sodium hydroxide solution and reduced over W-4 Raney nickel at 1,600–1,700 lbs./sq. in. (room temperature) at 150–200°. The yields, boiling points, and neutralization equivalents are listed in Table I.

Cyclohexylacetyl Chloride.—One-tenth mole of cyclohexylacetic acid was refluxed on a water bath for one hour with 22.5 ml. of purified thionyl chloride (13). After removing the excess thionyl chlo-

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TABLE I.—REDUCTION OF ACIDS CONTAINING AN AROMATIC RING

Starting Material	Composition (Reduced)	Boiling Point, ° C.	Yield, %	Neut. Equiv. Calcd.	Neut. Equiv. Found
Benzoic acid	$C_7H_{12}O_2$	138–140/2 mm.	83	128.2	128.2
o-Methoxybenzoic acid	$C_8H_{14}O_2$ sought		0	158.2	
	$C_7H_{12}O_2$ obtd.	138–140/2 mm.	70	128.2	129.1
Phenylacetic acid	$C_8H_{14}O_2$	158–161/45 mm.	80	142.2	142.6

ride under reduced pressure, 14.3 Gm. of cyclohexylacetyl chloride was collected at 103–105°/32 mm.

Veratroyl Chloride.—Seventeen and four-tenths grams of veratric acid (14) and 21 ml. of purified thionyl chloride were refluxed on a water bath for one and one-half hours. After removing the excess thionyl chloride under reduced pressure, 16.8 Gm. of veratroyl chloride was collected at 183–186°/34 mm. The acid chloride solidified on cooling.

Acetylvanillic Acid.—Seventeen grams of vanillic acid (15) was acetylated by the method of Chattaway (16). After cooling in an ice bath for five to six hours, 14 Gm. of acetylated acid was collected and dried. It was recrystallized twice from 50% ethanol to yield a product which melted at 146–147°.

Acetylvanilloyl Chloride.—Sixteen grams of acetylvanillic acid was heated on a water bath with 22.5 ml. of purified thionyl chloride. After removing the excess thionyl chloride under reduced pressure, three successive 15-ml. portions of dry benzene were added and distilled off to remove the remaining traces of thionyl chloride. The residual light brown oil was poured into an evaporating dish and cooled until it solidified. The crude acid chloride weighed 16.2 Gm., melted at 54–55°, and was used without further purification.

***p*-Methoxyphenylacetic Acid.**—This acid was prepared by the Kindler modification of the Willgerodt reaction (17), except for the following modification. After refluxing *p*-methoxyacetophenone, morpholine, and sulfur for twelve hours, the hot solution was poured onto 4 to 5 times its weight of ice and stirred until most of the ice had melted. Twice this volume of ether was added and stirred until the thiomorpholide crystallized. The crude product was collected in a Büchner funnel, washed with several portions of cold water, followed by two washings with cold ether. The product was air dried to yield 83% of the theoretical amount. Hydrolysis of the thiomorpholide yielded 44% (overall) of the theoretical amount of *p*-methoxyphenylacetic acid.

***o*-Methoxyphenylacetic Acid.**—*o*-Methoxybenzaldehyde was prepared by methylating salicylaldehyde in alkaline solution (18). The methoxyaldehyde was then mixed with hippuric acid, acetic anhydride, and sodium acetate and heated to form the azlactone (19) which was hydrolyzed and oxidized to *o*-methoxyphenylacetic acid (20). This acid was separated from benzoic acid by fractionating their methyl esters. Subsequent saponification yielded the acid which was recrystallized from benzene until it melted at 123–123.5°. The over-all yield from *o*-methoxybenzaldehyde was 45%.

γ-(*p*-Methoxyphenyl)-butyric Acid.—This acid was prepared by the reduction of β-(*p*-methoxybenzoyl)-propionic acid with zinc and hydrochloric acid (21).

Methoxyphenylaliphatic Acid Chlorides.—One-tenth mole of the acid and 22.5 ml. of purified thionyl chloride were allowed to stand together at room temperature for two hours, then heated at 50–60° on a water bath for thirty minutes. Most of the excess thionyl chloride was removed by distillation under reduced pressure. The remainder was removed by adding three successive portions of dry benzene and distilling each off under reduced pressure. The crude, oily acid chloride remaining was used immediately without further purification.

Acetoxymethyl Ketones.—The method of Elderfield and his co-workers (6) was used to prepare the desired ketones. Sixty-six thousandths mole of acid chloride, dissolved in dry ether or dry benzene (veratroyl chloride and vanilloyl chloride), was added dropwise to 300 ml. of a cold ethereal solution of diazomethane prepared from 30 Gm. of N-nitrosomethylurea. Stirring was continued for thirty minutes after the addition was completed. After standing overnight at room temperature, the ether was removed and the residue treated with 150 ml. of glacial acetic acid. The resulting solution was allowed to stand for several hours. It was then heated in a boiling water bath for thirty minutes, after which the excess acid was distilled off under reduced pressure. The residue was taken up with ether, washed with water, sodium bicarbonate solution, again with water and dried. After removing the ether, the ketone was distilled under reduced pressure. In the case of the solid ketones, the oily distillate soon crystallized and the product was recrystallized from an ether-petroleum mixture. The boiling points, melting points, yields, and analyses of the acetoxymethyl ketones are listed in Table II.

β-(3-Methoxy-4-hydroxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide—Eight and forty-five hundredths grams of 3-methoxy-ω-4-diacetoxyacetophenone, 4.5 Gm. of clean 30-mesh zinc and 70 ml. of dry, thiophene-free benzene were placed in a 500-ml., three-necked flask, equipped with a mechanical stirrer and a condenser set for downward distillation. After distilling off 20 ml. of benzene to remove any traces of water, the condenser was set in reflux position and fitted with a calcium chloride tube. The flask was then heated on a water bath and 3.6 ml. of freshly distilled ethyl bromoacetate added, all at once. If necessary, the reaction was initiated with a small crystal of iodine. After about twenty-five minutes, a benzene-insoluble oil began to separate and tended to stop the reaction. Heating and stirring were continued for fifteen minutes after the oil was first noticed. The stirrer was then removed and the benzene distilled off under reduced pressure. The residue was separated from the zinc with 45 ml. of 20% hydrochloric acid. The aqueous mixture was decanted from any remaining zinc into a beaker and heated on a water bath until all the

TABLE II.—ACETOXYMETHYL KETONES

$$RCCH_2OCCH_3$$
$$\begin{matrix} \| & \| \\ O & O \end{matrix}$$

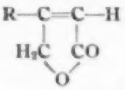
R	M. P., °C.	B. P., °C.	Yield, %	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3-Methoxyphenyl		174–178/11 mm.	67	63.45	63.20	5.81	5.86
3,4-Dimethoxyphenyl	82.5–83.5[a]	181–185/3 mm.	85	60.50	60.52	5.92	5.96
3-Methoxy-4-acetoxyphenyl	78–78.5[b]	197–202/3 mm.	73	58.64	58.76	5.29	5.36
Hexahydrobenzyl		110–117/3 mm.	62	66.64	66.18	9.15	9.02
p-Methoxybenzyl	61–62	178–181/5 mm.	84	64.83	64.95	6.35	6.38
o-Methoxybenzyl		150–156/3 mm.	66	64.83	64.24	6.35	6.31
γ-(*p*-Methoxyphenyl)propyl		237–241/35 mm.	72	67.18	66.89	7.25	7.29

[a] Recorded melting point 91–92° (22). [b] Recorded melting point, 73° (23).

material had dissolved. The clear solution was poured onto 200 ml. of ice and water and stirred until a powdery solid formed. The brownish powder was collected on a suction filter and, after drying, it was recrystallized from 95% ethanol to yield a white powder. The physical properties, yield, and analysis are listed in Table III.

ice and water. After the ice had melted, the oil was extracted with benzene. The benzene solution was washed with water, sodium bicarbonate solution, again with water, and dried. Removal of the benzene left a small residue which was distilled under reduced pressure. The distillate was dissolved in a small amount of benzene and poured onto a col-

TABLE III.—BETA-SUBSTITUTED $\Delta^{\alpha,\beta}$-BUTENOLIDES



R	M. P., °C.	B. P., °C.	Yield, %	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3,4-Dimethoxyphenyl	143–144		3	65.44	65.64[a]	5.49	5.49[a]
3-Methoxy-4-hydroxyphenyl	172–173		69	64.07	63.95	4.89	4.97
Hexahydrobenzyl	...	145–147/3 mm.	40	73.30	72.89	8.95	8.91
p-Methoxybenzyl	51–52	...	14	70.55	70.66[a]	5.93	5.71[a]
o-Methoxybenzyl	...	...	13	70.55	70.38	5.93	6.14
γ-(*p*-Methoxyphenyl)propyl	...	198–202/3 mm.	26	72.39	71.94	6.94	7.15

[a] Clark Microanalytical Laboratory, Urbana, Ill.

Beta-substituted $\Delta^{\alpha,\beta}$-butenolides.—Five-hundredths mole of acetoxymethyl ketone, 6.9 Gm. of 30-mesh zinc and 70 ml. of dry, thiophene-free benzene were placed in a 500-ml., three-necked flask equipped with a mechanical stirrer and a condenser set for downward distillation. After distilling off 20 ml. of benzene to remove any moisture present, the condenser was set in reflux position and fitted with a calcium chloride tube. Five and eight-tenths milliliters of ethyl bromoacetate was added, all at once, and heating and stirring begun. After the initial vigorous reaction subsided, the mixture was heated and stirred on a water bath for two hours. The mixture was then cooled and the benzene solution decanted onto 200 Gm. of ice and 20 ml. of concentrated hydrochloric acid. After the ice had melted, the mixture was transferred to a separatory funnel and shaken vigorously to decompose any zinc complex remaining. The organic layer was then separated, washed with water, sodium bicarbonate solution, again with water, and dried. After removing the benzene under reduced pressure, the residue was heated for thirty minutes at 120–130° in an oil bath with 30 ml. of glacial acetic acid saturated with hydrogen bromide. The warm solution was then poured, with stirring, into 200 ml. of

umn of aluminum oxide and eluted with 80 ml. of dry benzene. Upon evaporation, a light yellow oil remained which could be distilled under reduced pressure.

All the $\Delta^{\alpha,\beta}$-butenolides gave a positive Legal test (24).

The physical properties, yields, and analyses are listed in Table III. β-(*o*-Methoxybenzyl)-$\Delta^{\alpha,\beta}$-butenolide was not obtained in large enough quantities to obtain a boiling point range.

Ultraviolet Spectra.—The ultraviolet spectra for the $\Delta^{\alpha,\beta}$-butenolides were determined in absolute ethanol with a Beckman D. U. spectrophotometer using matched silica cells.

DISCUSSION

High-pressure reduction of aryl and aralkyl acids in alkaline solution with hydrogen over W-4 Raney nickel is an excellent method for preparing certain saturated cyclic acids. However, *ortho* and *para*-methoxy benzoic acids are demethylated under these conditions. Demethylation of these two acids without reduction was accomplished at atmospheric pressure by stirring Raney nickel alloy with an alkaline solution of the acids (25).

Since W-4 is more active than some of the other Raney nickel catalysts, such as W-2, by virtue of the presence of small amounts of aluminum metal, it is possible that aluminum may be responsible for the demethylation. Demethylation does not occur with the *meta* isomer (25). The rate of reduction of *o*-methoxybenzoic acid was much slower under high pressure conditions with W-4 than for benzoic acid under the same conditions or for the demethylation at atmospheric pressure with Raney alloy (25). This indicates that the demethylation step probably precedes the reduction step.

The difference in physical properties and ultraviolet spectra of the β-alkyl and β-aralkyl-$\Delta^{\alpha,\beta}$-butenolides and those of the β-aryl-$\Delta^{\alpha,\beta}$-butenolides is a result of the inability, respectively, of the aromatic rings to be conjugated with the double bond of the lactone ring. The alkyl and aralkyl derivatives are liquids with the exception of β-(*p*-methoxybenzyl)-$\Delta^{\alpha,\beta}$-butenolide, which is a low-melting solid. The β-aryl-$\Delta^{\alpha,\beta}$-butenolides are higher melting solids.

The ultraviolet spectra of the butenolides, Figs. 1 and 2, indicate the profound effect of conjugation between the aromatic groups and the lactone ring. Where no conjugation exists, the peak of the band of the methoxyphenyl ring is in same position and of the same intensity as that for anisole. However, when conjugation is possible, the absorption band for the aromatic ring is intensified and shifted toward the longer wavelengths. β-(3,4-Dimethoxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide and β-(3-methoxy-4-hydroxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide were strongly fluorescent under an ultraviolet lamp.

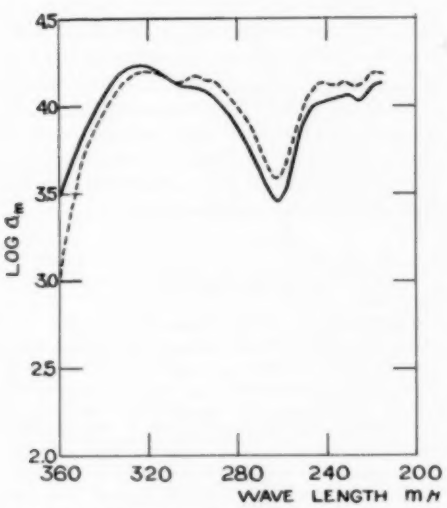


Fig. 1.—Absorption spectra of ——— β-(3-methoxy-4-hydroxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide, and – – – β-(3,4-dimethoxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide.

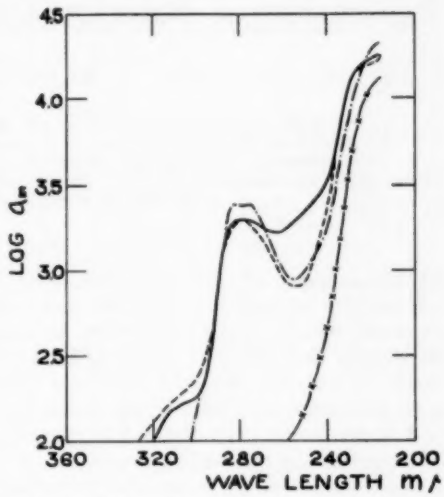


Fig. 2.—Absorption spectra of ——— β-(*p*-methoxybenzyl)-$\Delta^{\alpha,\beta}$-butenolide, —·— β-(*o*-methoxyphenyl)-$\Delta^{\alpha,\beta}$-butenolide, – – – β-[γ-(*p*-methoxyphenyl)-propyl]-$\Delta^{\alpha,\beta}$-butenolide, and —x— β-(hexahydrobenzyl)-$\Delta^{\alpha,\beta}$-butenolide.

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Evaluation of Solution Quality—Statistical Approach*

By JOHN J. HALSEY

A simple, practical test is presented by which the trace mechanical impurities in pharmaceutical solutions may be evaluated on a reproducible basis, thus making it possible for a producer and consumer(s) to have a mutual understanding of acceptable and nonacceptable quality. The theoretical basis for the test is given and the use of the data on a production control chart is outlined.

THE PROBLEM of evaluating the physical qualities of solutions of pharmaceutical products, especially parenterals, which are sold as solutions or to be made up in solution when ready for use, falls generally into three categories: (*a*) color, (*b*) clarity or conversely haze, and (*c*) mechanical impurities such as fragments of paper, fiber, or dust and specks.

Color of solution can be approached by either the use of physical color standards or by spectrophotometric methods. Clarity or haze problems may be evaluated and standardized by nephelometry. The last category, however, has not been amenable to quantitative measurement generally. The R. C. A. ampul-inspection machine (8) is being evaluated for testing materials already in solution.

This paper is concerned primarily with the evaluation of the so-called mechanical impurities of dry materials which are put in solution at the time of use. The approach, however, need not be restricted to this property.

In U. S. P. XIII (10) a method of examination of solutions was described and a qualitative restriction proposed, namely, that the solution be "substantially free of any turbidity or undissolved material." In the absence of a physical standard and an objective means of evaluation, the test was entirely subjective and presumably because of this was omitted from U. S. P. XIV (11). With respect to these mechanical impurities in solution, the mind is not capable of retaining a fixed mental standard without continual reference to a physical standard and some means of assigning a value to the results. This was brought out in the case of the F. D. A. *vs.* Bristol Labs. (4), which was dismissed (5) on the grounds of insufficient evidence (5) and indefiniteness of the official standard (10).

* Received July 12, 1954, from the Chemical Control Division, Merck & Co., Inc., Rahway, N. J.

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The aim of this paper is to outline a procedure for the examination of solutions and present a method for the objective evaluation of results. The theoretical background for the test is given. An example of the use of the data as a production tool (control chart) is outlined.

The mechanical impurities of concern are those known as fuzz, fibers, floaters, specks, or undissolved material, which includes fine, light particles, light flocculent or wooly masses, threads, threadlike materials, floating pieces, and dust. On an absolute scale of weight measurement these impurities are probably in the order of parts per million or less. In a solution of pharmaceutical material for which the test is especially designed, the number of mechanical impurities is small but not readily amenable to count because of motion, variety of sizes, and variable characteristics of the particles. However, since the quantity is small, multiple solutions prepared from an otherwise well blended solid material will not necessarily be exactly alike, but will differ a little one from another. This difficulty is overcome by examination of groups of solutions of the standard and sample as outlined under the procedure below.

PROCEDURE

The Standard.—The primary standard is a physical sample of well blended material which is acceptable to the interested parties. These parties may be represented by the various departments within a company; a supplier and customer(s); or some broader base of interests. The standard should be tested against itself by the same procedure used for the subsequent evaluation of samples against the standard to establish that it is sufficiently uniform for the purpose. These prepared solutions of the standard are also shown to the interested parties and are the basis on which agreement to the primary standard is reached.

The samples and standard should be made up to the same concentration in fuzz-fiber-floater free distilled water in thoroughly cleaned vials free of fuzz, fibers, and floaters.

Preparation of Standards for Test.—Eight individual 5-Gm. samples of the primary standard are each dissolved in 25 ml. of water in 50-cc. serum

vials and closed with a suitable stopper. If necessary a drop of phenol may be added to each vial to prevent growth of molds.[1]

Preparation of Sample for Test.—Samples of material to be evaluated are prepared in exactly the same manner as the standard, i. e., in this case eight individual solutions.

Light Source and Background for Observation.—A suitable device for observing the quality of solutions is a gooseneck desk lamp placed in front of a vertical screen. The desk lamp should be provided with hemispherical or other suitable type shade preferably lined with frosted white to minimize reflection of images and provided with a 100-watt incandescent bulb. The screen should have a dull black finish. The examination should be made in a darkened room or in a large cubicle with a black inside finish to reduce the distractions of light reflections.

Examination and Ranking.—The lamp shade should be tilted to protect the observer's eyes from direct illumination. A vial is picked at random by the observer or tester from the 16 vials of solutions (8 standards, 8 samples) and is twirled slightly to rotate the solution contents, and while the solution is gently swirling it is held in position a few inches below the front edge of the light source and examined. This examination is repeated for a second vial and the observer decides whether this second vial is better or worse than the first. This examination is repeated for the third vial picked at random and the observer ranks the third vial relative to the preceding two vials. The remaining vials are examined in like manner, and the observer ranks all 16 in order of goodness, regardless of whether they are standards or samples, from 1 to 16. The best solution (least fibers, fuzz, floaters) will be ranked 1, the worst will be number 16. We have found it convenient to do a quick gross ranking on the first series of observations, then refine the ranking by a second and sometimes a third series of examinations. Little is gained by attempting to get greater perfection by more examinations than this because slight differences in the order of ranking do not usually lead to different decisions in regard to disposal of the product in question.

If two (or more) solutions of either standard or sample appear exactly alike, assign a mean rank to them (e. g., suppose of the 16 solutions, 1 through 9 have been ranked, but the next two appear so nearly alike it is not possible to decide which is to be ranked 10 and which 11, in this case the observer should assign a mean rank of 10.5 to each of the two vials in question).

Evaluation of Results and Action.—A typical ranking shows the following:

Rank of best to worst:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Identity of the vials:	*St*	*Sa*	*Sa*	*St*	*Sa*	*St*	*St*	*Sa*	*Sa*	*Sa*	*St*	*St*	*Sa*	*Sa*	*St*	*St*

Where *St* = a vial of the standard (of which there are eight).
Sa = a vial of the sample (of which there are eight).

Tabulate the values for the standard and the sample and take the sum of the respective ranks.

Sum for Standard:

$$1 + 4 + 6 + 7 + 11 + 12 + 15 + 16 = 72$$

Sum for Sample:

$$2 + 3 + 5 + 8 + 9 + 10 + 13 + 14 = 64$$

Depending on the sum of the ranks, three alternative courses of action may be taken: (*a*) If the sum of the results for the standard is equal to or less than 49 (the sample is 87 or more), then the sample is to be rejected as being significantly worse than the standard; (*b*) if the standard is greater than 49 but less than 87 (sample less than 87, greater than 49), the sample is not significantly different from the standard (this is the case illustrated above); and (*c*) if the sum for the sample is less than 49 the sample is significantly better than the standard.

THEORETICAL BACKGROUND FOR THE TEST

The approach used in this paper was suggested by the published work of Wilcoxon (12) but differs in that the derivations are more specific and the distinction between the characteristics of the universe and of the sampling procedure from that universe are clearly shown, which makes the theory more easily understood, at least to this writer. In the abstracted table of probabilities shown below the one- and two-sided risks are indicated.

The frequency distribution of the individual ranks would be rectangular in shape and would be the same type, for example, as the distribution of spots on a single die (3). The mean (μ) of such a rectangular distribution would be one half the range (h) of the ranks, and the variance (σ^2) would be $h^2/12$ (1). For this problem, however, we are interested in knowing whether there is a significant difference between the standards and the samples and this is done by means of rank sums, rather than by individual ranks. The frequency distribution of rank sums would approach a normal distribution as the number of samples of n increased (or as repetitions of the ranking procedure were made on the same samples).

Development of Formula for "Expected" Sum.—Let x_i be an individual rank value. (In the case under study here x_i may take on any value from 1 to 16.) Then the sum of the ranks for a sample of eight would be $x_1 + x_2 + x_3 + x_4 + x_5 + x_6 + x_7 + x_8$. This may be represented, if n is the number of sample items and S is the sum, by the equation

$$S = \sum_{1}^{n} x_i$$

The mathematically expected rank sum (ES) for the samples (or conversely for the standards), if there were no differences in the standards and sam-

[1] These concentrations, vial size, and preservatives were found suitable for our investigation, but may be changed to any other suitable conditions for any particular problem. The procedure may thus be made quite general.

ples, is the average of all possible sample results. The total number of possible combinations of samples of (n) out of a total (N) is given by the combinatorial formula $\binom{N}{n} = N!/(N-n)!\,n!$

The formula for the "expected" rank sum would be by definition:

$$ES = \frac{\sum_{1}^{\binom{N}{n}} \sum_{1}^{n} x_i}{\binom{N}{n}}$$

To simplify this formula for practical use, since there are $\binom{N}{n}$ possible samples of n, each sample containing n/N of the rank values, any given rank (x_i) may appear $\binom{N}{n}\frac{n}{N}$ times in the possible ranks of the sample solutions, thus

$$ES = \frac{\binom{N}{n}\frac{n}{N}\sum_{1}^{N} x_i}{\binom{N}{n}} = \frac{n}{N}\sum_{1}^{N} x_i$$

But in the case of equal number of standard solutions and sample solutions, $n/N = 1/2$ and $\sum_{1}^{N} x_i$ is the sum of an arithmetic progression from 1 to N which is equal to $N(N+1)/2$, and this gives the simplified formula for the expected rank sum as:

$$ES = \frac{N(N+1)}{4}$$

Development of Formula for Variance.—The general formula for variance of a universe of N finite numbers,[2] $x_1, x_2, \ldots, x_N$ and having a mean ($\mu = \sum x_i/N$) is, by definition:

$$\sigma^2 = \frac{1}{N}\sum_{1}^{N}(x_i - \mu)^2 = \frac{1}{N}\left[\sum_{1}^{N} x_i^2 - \frac{\left(\sum_{1}^{N} x_i\right)^2}{N}\right]$$

(an algebraic identity.)

As in the case of the derivation of the formula for ES above, $\sum_{1}^{N} x_i$ is the sum of an arithmetic progression from 1 to N and $\sum_{1}^{N} x_i^2$ is a series from 1 to N^2. Substituting in the above formula for variance gives:[3]

$$\sigma^2 = \frac{1}{N}\left[\frac{N(N+1)(2N+1)}{6} - \left(\frac{N(N+1)}{2}\right)^2 \cdot \frac{1}{N}\right] = \frac{N^2-1}{12}$$

From here, the development depends on the *sampling procedure* from the frame of N items. The samples (n) are drawn at random without replacement. The formula for the variance of $\bar{x}$ under these conditions is:[4]

$$\sigma_{\bar{x}}^2 = \frac{N-n}{N-1}\frac{\sigma^2}{n}$$

$(N-n)/(N-1)$ is the finite multiplier and corrects for the finite size of the universe when the sampling is done without replacement. The sum (S) for the sample is $\sum_{1}^{n} x_i$ or equivalently $S = n\bar{x}$ and the variance for this sum is:

$$\sigma_s^2 = n^2 \cdot \frac{N-n}{N-1}\frac{\sigma^2}{n} = n\,\frac{N-n}{N-1}\,\sigma^2$$

In the test described, $N = 16$, $n = 8$ or $2n = N$. The σ^2 for the universe was shown above to be $(N^2-1)/12$. Substituting gives:

$$\sigma_s^2 = \frac{N}{2}\cdot\frac{(2N-N)}{2(N-1)}\cdot\frac{(N^2-1)}{12} = \frac{N}{12}\cdot\frac{N(N+1)}{4}$$

It was shown in the previous derivation that $N(N+1)/4 = ES$ and substituting gives the simplified formula:

$$\sigma_s^2 = \frac{N(ES)}{12}$$

Theoretical Basis for the Courses of Action Outlined under Procedure.—The expected sum for the samples as described under the procedure above using the formula developed for ES is

$$ES = \frac{N(N+1)}{4} = \frac{16(16+1)}{4} = 68$$

The variance for the method outlined under the procedure using the formula developed for variance is

$$\sigma_s^2 = \frac{N(ES)}{12} = \frac{16(68)}{12} = 90.6$$

The standard error of S is the square root of the variance

$$Std.\ Error = \sqrt{90.6} = 9.5$$

The 95% confidence limits for test results from the expected value ES for the approximately normal

[2] The universe may more appropriately be characterized by $a_1, a_2 \ldots a_N$ and x_1, x_2, etc., reserved for the sample characteristics, but x has been used here to simplify the relation of this derivation to the preceding derivation for ES.

[3] The variance for a continuous rectangular distribution is equal to $h^2/12$ (Deming (1)); the "minus one" in this formula is due to the fact that the formula is for a discrete rectangular distribution, but since the square of N is involved the "minus one" would not usually make much practical difference.

[4] See page 102 Deming (1).

distribution of the sums, on the null hypothesis of no difference from ES, is:

$$ES \pm 1.96\sqrt{N(ES)/12} = 49 \text{ or } 87$$

This is the explanation for the values listed under the procedure on which action is based. Wilcoxon (12) gives probability tables for the 0.05, 0.02, and 0.01 levels for $n = 5$ to $n = 20$. A modified (as to one- or two-sided risks) and abstracted form of one of Wilcoxon's tables (12) (his Table I, p. 121) is shown in Table I of this paper.

TABLE I.—APPROXIMATE PROBABILITY THAT A VALUE FOR THE RANK SUM (S) OF THE SAMPLE WILL BE LESS THAN THE TABULATED VALUE IN THE BODY OF THE TABLE BY CHANCE[a]

Sample size n	Probability (One-Sided Risk) $P = 0.025$	$P = 0.01$	$P = 0.005$
6	27	24	23
7	37	34	32
8	49[b]	46	44
9	63	59	56
10	79	74	71
	$P = 0.05$	$P = 0.02$	$P = 0.01$
	Probability (two-sided risk)[c]		

[a] The probability values are based on the normal integral since the rank sums follow a reasonably normal distribution. For samples as small as those shown, the true risk is either very slightly less than or greater than the P values in this table. Exact probabilities are given by von der Vaart (13).
[b] This is the value calculated in the text above.
[c] That the sample is significantly *greater* or *less* than the standard. The tabulated value then applies to the rank sum for the sample or the standard, whichever is lower, and the probability shown at the bottom of the table is used.

DISCUSSION OF RESULTS

The sampling distribution of rank sums ($S = n\bar{x}$) of samples of eight in these experiments was approximately normal and followed a pattern similar to that for means ($\bar{x}$) of samples of eight from a rectangular distribution illustrated by Dixon and Massey (2).

Reproducibility of the Rank Scores.—In a series of tests, operators testing the same samples tend to get the same rank scores, (i. e., a difference of zero). Most of the time two operators agreed within 4 units or less and very infrequently did two operators disagree by as much as, or more than 12 units.

Correlation of the Actual Ranks Obtained by Two Operators.—If there were no correlation in the rankings by two operators (i. e., that the operators' rankings are unrelated) the values for the rank correlation coefficient would tend to cluster about zero. If, however, the value of the correlation coefficient is +1 there is perfect agreement and −1 perfect disagreement (or negative correlation). We may regard $R = 0$ as a null hypothesis. If our value of R appears then in the "tail" of the distribution of R, the null hypothesis is rejected. If in applying the t-test we find probability of 0.05 or 0.01, we may say the result is probably significant or significant; values of P of 0.001 we may say are highly significant. If it is expected that there will be a positive correlation before the t-test is made, as is the case in this study, one may use a "one-tail test." For detailed analysis of rank correlation methods see Kendall (7).

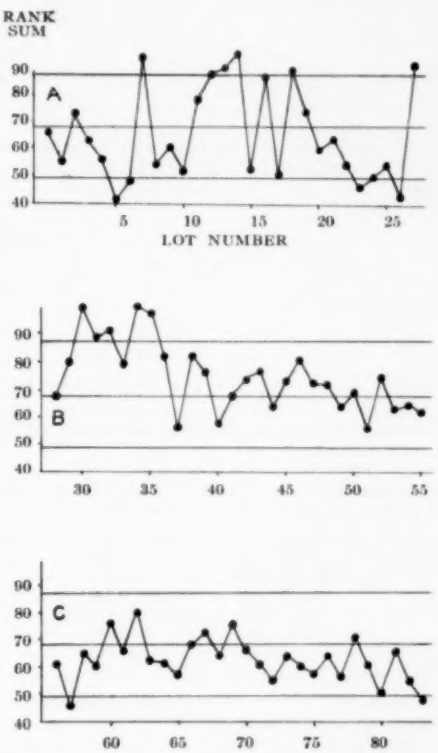

Fig. 1.—A chart showing the values of the rank sums obtained from samples of lots of material from a production process. The center line is the expected sum ES, the upper control limit is $ES + 1.96\ \sigma_s$, the lower limit is $ES - 1.96\ \sigma_s$. (A) represents process before control was achieved, (B) process stabilized and in control, and (C) process improved significantly (low values represent best quality).

In order to determine if analysts agreed in the individual ranking of the vials in a series, each vial in a complete test was identified by a letter (a through p) and the particular rank given to each vial (1 through 16) by analyst A and by analyst B were recorded. Spearman's rank correlation coefficient which is defined by:

$$R = 1 - \frac{6\sum d^2}{N^3 - N}$$

where $\Sigma\ d^2$ is the sum of the squares of the rank differences and N is the number of vials ranked, was calculated as shown in the following example:

CALCULATION OF RANK CORRELATION COEFFICIENT

Vial	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*
Analyst *A*	1	7	9	10	2	3	8	11	6	5	4	16	15	13	14	12
Analyst *B*	1	7	8	5	3	4	9	13	14	10	2	16	12	15	11	6
Rank difference (*d*)	0	0	1	5	−1	−1	−1	−2	−8	−5	2	0	3	−2	3	6
d^2	0	0	1	25	1	1	1	4	64	25	4	0	9	4	9	36

$$R = 1 - \frac{6(184)}{4,080} = +0.73$$

The significance of this value may be evaluated for N-2 degrees of freedom by use of Student's t-test.

Where

$$t = R\sqrt{\frac{N-2}{1-R^2}}$$

In this sample

$$t = 0.73\sqrt{\frac{14}{1-0.53}} = 4.03$$

This result is highly significant and we may conclude that there is an excellent correlation in the results obtained by two operators.

The subjointed table lists several typical rank correlation coefficients and t-values for the results of a series of ranking by two operators.

Typical Rank Correlation Coefficients, R	t Value
+0.61	2.87
+0.73	4.03
+0.83	5.58

The typical results shown in this table indicate significant correlation.

Why Use Eight Samples?—As noted in the introduction, multiple solutions are necessary for the comparisons because the solutions prepared from a blended solid differ a little one from another.

There are two limitations to the number of samples compared. A lower limit because of theoretical restrictions: with samples of 5 it is not possible to establish a significant correlation at the $P = 0.01$ level as demonstrated by Snedecor (9). Also by the method of calculation outlined under the preceding section *Theoretical Basis for the Courses of Action*, $-2\,\sigma_s$ limits for samples of 5 would be 15.9 and the complete rank separation in a test of 5 samples and standards would give a minimum rank sum of 15. Thus to make a decision at the $P = 0.05$ level would require all the samples to have rank values less (or all greater than) the standard vials.

The upper limitation to the number of sample vials to be ranked is based on practical considerations. If one has 10 samples and 10 standards, it would require the ranking of 20 vials and if the samples and standards were reasonably alike in quality, it would be time consuming, cumbersome, and difficult to establish the ranks for this large number of vials.

Plotting Results of Rank Sums on a Control Chart.—While the original evaluation of samples in comparison with the standard consisted of visual comparisons, the results (rank sums) are expressed as a number and this number has properties which can be handled in the same manner as a quantitative result (albeit not with as great precision as an original quantitative measurement, but with far greater effect than a purely subjective evaluation).

The results may be put on a control chart using ES as the center line and the upper and lower control limits as $\pm 1.96\sqrt{N(ES)/12}$ since the distribution of rank sums tends to a normal distribution.

The results of such a plot of results from a production process are shown in Fig. 1.

It is noted that when this control technique was first instituted the process was in a poor state of control (Fig. 1, points 1 to 27), the values fluctuating widely with points out of control on both sides (5 on the upper, 4 on the lower).

In Fig. 1*B*, the first 9 points continue to show the wide fluctuations on the high side (poor quality) but then a stabilizing influence and a clustering about the expected value (similarity to the standard) follows.

In Fig. 1*C*, the process is definitely improved showing reasonably good control, and a definite tendency to appear better than the standard, which is desirable. At this point it might be advisable to use a new standard.

From the charts one may observe several "runs," e. g., points 70 through 77 inclusive, which is significant. If there were no difference in the samples and the standard, one would expect a random distribution about the expected value of 68. The chance of getting a value on one side of the expected value is 0.50 if the values are random; of getting two values on one side is $(0.50)^2$ of n values is $(0.50)^n$ (Deming (1), p. 127).

A simple calculation on the probability of a run of 8 points all above ES (or all below) is $(0.50)^8$ or 0.004. This then could happen by chance only about one time in 250 and since this is improbable, we must conclude the production is, at least at this point, significantly better than the standard. Similarly points 29–36 represent production worse than the standard. [See also Duncan (2) on sampling distribution of runs; also Hoel *Introduction to Mathematical Statistics* (5)].

SUMMARY

1. A test has been described which makes it possible to evaluate solution quality on a reproducible basis and minimize difficulties due to differences of subjective opinion between departments with a company, between a supplier and customer(s) or some broader base of interests.

2. Rules for action are outlined as well as the theoretical basis for these rules. Sufficient information is given so that the test may be adapted to any particular circumstances.

3. The reproducibility of the test, a measurement of the significance of the results, and limitations of the technique are outlined.

4. The value of the test as a production tool (control chart) is indicated.

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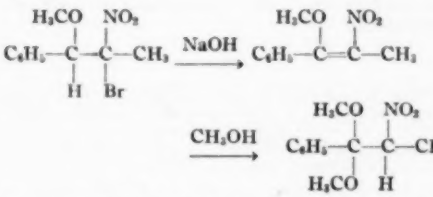
Colorimetric Determination of 2-Nitro-2-bromo-1-methoxy-1-phenylpropane*

By LAWRENCE R. JONES and JOHN A. RIDDICK

A method is presented for the colorimetric determination of 2-nitro-2-bromo-1-methoxy-1-phenylpropane in lotions and ointments containing 0.5 or 1.0 per cent of the active ingredient. The method is rapid and has an accuracy of ±5 per cent with a precision of ±2 per cent. Amounts as little as 25–50 μg. can be determined.

2-NITRO-2-BROMO-1-METHOXY-1-PHENYLPROPANE is effective as a fungicidal agent (3). A colorimetric method of analysis has been developed which is applicable to various mixtures and pharmaceutical preparations. This nitro-bromo compound can be hydrolyzed in an alcoholic alkaline medium to form a 2-nitro phenylalkane. The reaction is:



Jones and Riddick (1) have shown that 2-nitro-1,1-bis(*p*-chlorophenyl)alkanes react with acidified iron (III) chloride solution to form a colored complex suitable for quantitative measurement. The 2-nitrophenylalkane obtained from the alkaline hydrolysis of the nitro-bromo compound reacts in a similar manner. The method of analysis is based upon the fact that a nitro group attached to a primary or secondary alkane carbon exists in equilibrium with its tautomeric aciform: $R_2CHNO_2 \rightleftarrows R_2C{=}NO_2H$. The equilibrium is shifted to the right in the presence of alkali. The aci-nitroalkanes form the colored complexes with iron (III) ion.

* Received February 4, 1954, from the Research and Development Department, Commercial Solvents Corporation, Terre Haute, Ind.

EXPERIMENTAL

Reagents.—(*a*) Standard 2-nitro-2-bromo-1-methoxy-1-phenylpropane, purified by recrystallization. (*b*) Butanol, reagent grade. (*c*) Ethanol, anhydrous, S. D. Formula 3A. (*d*) Methanolic sodium hydroxide, 0.500 *N*. (*e*) Iron (III) chloride reagent, 10 Gm. of iron (III) chloride, $FeCl_3 \cdot 6H_2O$ dissolved in sufficient water to make 2,000 ml. of solution and mixed with 1,000 ml. of 0.600 *N* methanolic hydrochloric acid. (*f*) Methanolic hydrochloric acid, 0.600 *N*.

Apparatus.—(*a*) Spectrophotometer, Beckman Model DU. (*b*) Tubes, Lewis-Benedict, Corning No. 7860, calibrated at 12.5 and 25 ml. (*c*) Steam bath, any type. (*d*) Water bath, cooling; any type.

Procedure.—The method consists of two steps, the hydrolysis of the compound and the formation of the colored complex. The effect of several variables were examined for their influence on the hydrolysis, color formation, and quantitative applications of the method.

Hydrolysis of Compound.—Butanol is the most satisfactory solvent for the hydrolysis. Several aliquots of sample containing 2.0 mg. of 2-nitro-2-bromo-1-methoxy-1-phenylpropane dissolved in butanol were treated at room temperature and at 100° with methanolic sodium hydroxide solution. The hydrolysis was followed by means of the color produced with iron (III) chloride. The results are shown in Table I. A ten-minute reaction time at 100° was chosen to insure complete hydrolysis.

TABLE I.—HYDROLYSIS OF 2-NITRO-2-BROMO-1-METHOXY-1-PHENYLPROPANE AT ROOM TEMPERATURE AND 100°C, 2-MG. SAMPLES

Room Temperature		100°C	
Time	Optical Density	Time, min.	Optical Density
10 min.	0.005	2	0.375
30 min.	0.060	4	0.555
2 hr.	0.290	6	0.660
8 hr.	0.625	8	0.715
12 hr.	0.715	10	0.715

Color Formation.—The absorption spectra, the effect of water on the colored complex, the effect of acidity on the intensity of color, the reaction time and temperature, and the validity of Beer's law were compared and found to coincide with the results obtained and reported for the determination of 2-nitro-1,1-bis(*p*-chlorophenyl)alkanes (1).

Color Stability.—The color of the iron (III) complex with 2-nitro-1,1-bis(*p*-chlorophenyl)alkanes (1) was found to be stable for one hour. The color of the iron (III) complex with the hydrolysis product of 2-nitro-2-bromo-1-methoxy-1-phenylpropane was found to be stable for only five minutes. This limited the preparation of only one sample of the iron (III) complex at a time.

Specificity of Method.—The measurement is made on the hydrolyzed derivative of the active fungicide. The specificity is obtained by measurement of the difference between the optical densities of the sample prepared by a ten-minute reaction at room temperature. It is shown in Table I that hydrolysis of 2-nitro-2-bromo-1-methoxy-1-phenylpropane is completed at 100° in eight to ten minutes while the reaction for a similar length of time at room temperature indicates no appreciable hydrolysis of the fungicide. It has been shown (1) that 2-nitrophenylalkanes (a similar type compound to the hydrolysis product 2-nitro-2-bromo-1-methoxy-1-phenylpropane) reacted completely within ten minutes at room temperature under similar test conditions.

Preparation of Calibration Curve.—Prepare a solution of 2-nitro-2-bromo-1-methoxy-1-phenylpropane in butanol to contain 1 mg./ml. To five Lewis-Benedict tubes add, respectively, 0.0, 0.5, 1.0, 1.5 and 2.0 ml. of the above standard solution and dilute to 5 ml. with butanol.

Add 1.0 ml. of methanolic sodium hydroxide, mix, and immerse the tubes in the steam bath for ten minutes. Remove and cool in the water bath.

Add 3.0 ml. of iron (III) chloride reagent to the first tube and mix. Dilute to 12.5 ml. with ethanol and mix. Transfer the solution to a 1-cm. Corex cell and read the optical density at 490 mμ using the 0.0 prepared standard as the blank for the zero optical density setting of the instrument. Repeat this procedure with the other solutions.

Plot concentration *vs.* optical density on linear graph paper. The above standards equal 0.0, 0.5, 1.0, 1.5 and 2.0 mg., respectively.

Analysis.—Make the analysis as described above, but prepare the sample so that a 5.0-ml. aliquot of the butanol solution contains not more than 2.5 mg. of 2-nitro-2-bromo-1-methoxy-1-phenylpropane.

Prepare a blank from a second 5.0-ml. aliquot of the sample by allowing a ten-minute reaction time at room temperature in the presence of the methanolic sodium hydroxide solution. The blank will correct for any decomposition of the active ingredient caused by slightly alkaline conditions such as is found in the alkaline pharmaceutical preparations.

ANALYSIS OF OINTMENTS

Determination in Emulsion Type Ointments.—The emulsion type ointments that were encountered in this laboratory were completely soluble in butanol. Weigh a sample of the ointment into a volumetric flask and after dilution to volume with butanol, assay a 5.0-ml. aliquot containing 2.5 mg. or less.

Determination in Carbowax Base Ointment.—The Carbowax® base ointments are not completely soluble in butanol, but the solvent extracts the 2-nitro-2-bromo-1-methoxy-1-phenylpropane from the ointment base. Weigh a sample of the ointment into a volumetric flask, dilute to volume with butanol, mix, and centrifuge. Analyze 5.0-ml. aliquots of the supernatant solution containing 2.5 mg. or less.

Determination in Petrolatum Base.—It is necessary to extract the 2-nitro-2-bromo-1-methoxy-1-phenylpropane from petrolatum ointments prior to analysis. This may be done by the two-phase *n*-hexane-acetonitrile separation technique described by Jones and Riddick (2). After the acetonitrile is evaporated, the residue is dissolved in butanol, diluted to volume with butanol, and a 5.0-ml. aliquot containing 2.5 mg. or less is analyzed.

Results.—Ointments of the three base types were prepared and analyzed. Typical data are presented in Table II.

TABLE II.—ANALYSIS OF OINTMENTS OF KNOWN COMPOSITION FOR 2-NITRO-2-BROMO-1-METHOXY-1-PHENYLPROPANE

Calculated, %w	Found, %w
1.00	0.95
1.00	0.98
2.00	2.03
2.00	1.99
0.50	0.48
0.50	0.51

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The Melting Point Behavior of *l*-Arterenol *d*-Bitartrate Monohydrate*

By ROBERT L. CLARKE†

The presence of seed crystals of anhydrous *l*-arterenol *d*-bitartrate in a laboratory makes it difficult to prepare samples of *l*-arterenol *d*-bitartrate monohydrate of melting point 102–104° as reported in the literature. These seed crystals cause partial resolidification as the anhydrous salt, m. p. 158–159° (dec.), at the melting point of the hydrate, partial decomposition of the melted material occurring simultaneously. These two factors, operating in varying degree, cause the melting range of the hydrated salt to extend from 100° upward toward that of the anhydrous salt.

THE RESOLUTION of *dl*-arterenol, α-aminomethyl-3,4-dihydroxybenzyl alcohol, has been reported by Tainter, Tullar, and Luduena (1) and Tullar (2) utilizing the *d*-bitartrate salt for the separation. *l*-Arterenol *d*-bitartrate monohydrate is listed in New and Nonofficial Remedies (3) as "Levarterenol Bitartrate" and is a commercial product under the tradename Levophed® bitartrate.[1]

Tullar (2) reports that this hydrated salt melts at 102–104° (corr.) and New and Nonofficial Remedies (3) gives a range of 100–106° in its specifications. Recently, samples of this compound, prepared in the laboratories of Winthrop-Stearns Inc.,[2] began to show a strange behavior when their melting points were determined; melting started at about 101° but cloudiness or solid persisted up to the 130–150° range. Here we are reporting the causes for this phenomenon.

EXPERIMENTAL

The salt in question crystallizes beautifully from water (Fig. 1) as long, orthorhombic prisms: optically (+); $2V = 85° \pm 2°$ (calcd.); elongation (−); Ng = 1.620, Nm = 1.577; Np = 1.531 (values ± 0.002).[3] Twinning is common but is apparent under polarized light only from the end view.

When this salt is heated on a microscope hotstage, melting begins at about 100° and, on samples of satisfactory melting point, is complete at about 104°. With samples showing the extended melting range, partial crystallization of the melt is observed as shown in Fig. 2 (under polarized light with crossed nicols). The degree of resolidification varies with the number of "seeds" of the high-melting solid in the particular sample, for unless crystallization occurs within a few seconds, it never occurs. This failure to crystallize is the result of a certain amount of decomposition within the melt. As evidence of decomposition, we have observed that material heated for three minutes at 120° drops in specific rotation from −11.3° to −5.4°. Yet the resolidified material is relatively stable at 120°. A sample, heavily seeded with the high-melting solid and heated for one minute at 115°, shows no apparent melting at that temperature but melts at 147–150° (dec.);[4] $[\alpha]_D^{24} -12.5° \pm 0.2°$ (2% in H_2O).

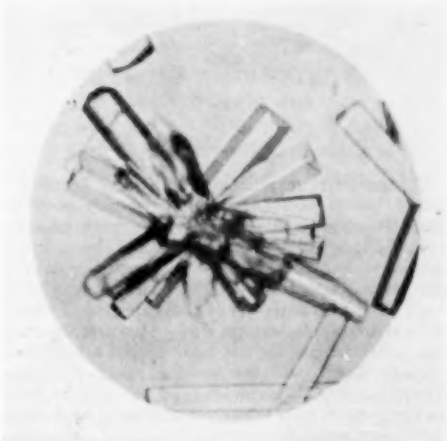

Figure 1.

This material was identified as anhydrous *l*-arterenol *d*-bitartrate.

Anal.—Calcd. for $C_{12}H_{19}NO_{10}$: C, 45.20; H, 5.33; N, 4.40. Found: C, 44.97; H, 5.28; N, 4.60; H_2O by Karl Fischer method, none; rotation expected from dehydration, $[\alpha]_D^{24} -12.2°$.

l-Arterenol *d*-bitartrate was prepared from its monohydrate salt by warming 19 Gm. of the latter in 150 ml. of methanol at 35° to achieve solution and allowing this solution to stand at 25° for three hours. The precipitated anhydrous salt was washed with a little methanol and ether and dried *in vacuo* over calcium chloride. The white needles (Fig. 3) (13.5 Gm., 75%) melted at 158–159° (dec.) (corr.); $[\alpha]_D^{25} -11°$ (1.6% in H_2O).

Anal.—Calcd. for $C_{12}H_{19}NO_{10}$: C, 45.20; H, 5.33; N, 4.40. Found: C, 45.60; H, 5.42; N, 4.27.

* Received April 14, 1954, from the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

† I wish to thank Miss E. Angell, M. E. Auerbach, R. S. Browning, F. Coulston, F. C. Nachod, and B. F. Tullar for assistance with this work.

[1] Levophed®, trademark reg. U. S. pat., Winthrop-Stearns Inc.

[2] Winthrop-Stearns Inc., New York, N. Y.

[3] These values are in good agreement with those reported by George L. Keenan in a private communication.

[4] This will vary by several degrees in different preparations.

Figure 2.

Figure 3.

Comparative dehydrations run on a "standard sample" of m. p. 101–103° and a sample of m. p. 101–130° showed that heating for twenty minutes at 81–84° and 0.3 mm. pressure caused the standard sample to lose 13% of its total moisture, whereas the "seeded sample" lost 64%. There seems to be some sort of potentiation of the water loss if nuclei of anhydrous solid are present. However, heating this latter sample for one hour caused a total loss of only 73.5% and the melting range was then 90–130° (dec.). Dehydration of the salt by heat and reduced pressure does not appear to be a practical procedure.

It appears that once seeds of the anhydrous *l*-arterenol *d*-bitartrate are present in a laboratory, it is very difficult again to prepare there the monohydrate with a melting range of 100–106°. In addition, the tendency toward decomposition seems to exclude dehydrative procedures for preparation of a material of sharp melting point. The losses on dehydration by the methanol method as described are serious. Thus it appears desirable to place more reliance on tests other than the melting point of *l*-arterenol *d*-bitartrate monohydrate in the specifications for this material.

SUMMARY

1. The recently observed increase in melting point for *l*-arterenol *d*-bitartrate monohydrate from the range 102–104° to the range 101° upward toward 150° is described and attributed to partial resolidification of the sample in the anhydrous form which melts at 158–159° (dec.).

2. The melting point of the hydrate becomes of limited value as a definitive physical constant for this compound since decomposition accompanies the dehydration and wide and nonreproducible melting ranges may be found.

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The Influence of Suppository Bases Upon the Rectal Absorption of Acetylsalicylic Acid*

By ANTHONY F. CACCHILLO† and W. HOWARD HASSLER‡

Plasma levels of acetylsalicylic acid were administered rectally in three different types of suppository bases and were compared to plasma levels of aspirin administered orally. Statistically no significant difference existed between the absorption of acetylsalicylic acid from the Carbowax®, type base given rectally and acetylsalicylic acid administered orally. Irritation studies conducted reveal that little or no irritation results from administration of acetylsalicylic acid suppositories of both the cocoa butter and Carbowax®, types, while glycerinated gelatin causes a high incidence of irritation.

A convenient form of drug therapy in use today which enjoyed widespread popularity as far back as the time of Hippocrates is the suppository. Through the years it has been used in medicine, serving a valuable function in drug absorption, particularly when other routes of administration were lacking or unsuccessful.

The use of suppositories may be indicated for many and varied reasons. If the drug is not absorbed by the mucous membranes it is used advantageously for the treatment of local conditions of the rectum, urethra, and vagina. When systemic action is desired, suppositories are indicated in patients where continuous emesis is present, when the patient is unable to swallow capsules or tablets, or is in a comatose condition. In the case of infants and children, when the oral route presents a problem, suppositories are particularly well suited. Some drugs rendered inactive by digestive fluids of the stomach or intestine, or which interfere with digestive functions, exert systemic effects when administered rectally.

It must be remembered that no set rule exists concerning the relative ease of absorption of drugs by the rectum, but that individual studies must be undertaken to determine for each drug the base best suited for its absorption.

From time to time reports have appeared in the literature that compared the absorption of medicaments when given orally and rectally. Tornack (1) reveals that the absorption of glucose from the rectum is negligible; while Blume and Nohara (2) employing rabbits, show that the absorption of sodium salicylate is greater rectally than orally. In a comparative study (3) six drugs were tested for absorption in sixty-three normal individuals. Five of these six drugs, *viz.*, sodium salicylate, chloral hydrate, methylene blue, atropine sulfate, and morphine sulfate, were absorbed in therapeutic quantities more quickly rectally than orally. Still another investigation (4) revealed that higher concentrations of salicylate in the blood of human patients were obtained by rectal instillation. Thus, these absorption studies make apparent the individuality of drug absorption when the rectal route is employed.

EXPERIMENTAL

In view of the recent interest shown in the rate of absorption of drugs from different suppository bases (5–11), we have evaluated the absorption of acetylsalicylic acid from three different types of suppository bases. As a basis for comparison, the aspirin plasma level following oral administration was assigned the arbitrary value of 100%. For this study, three common suppository bases available today were used, *viz.*, cocoa butter,[1] glycerinated gelatin, and a mixture of the polyethylene glycols.

Suppositories composed of a glycerinated gelatin base were prepared by melting glycerinated gelatin on a water bath and incorporating the appropriate amount of acetylsalicylic acid. The mixture was poured into molds and cooled at room temperature until solidified. A suitable Carbowax® suppository base was formulated, consisting of 40% Carbowax® 6000, 30% Carbowax® 1540, and 30% polyethylene glycol 400. The Carbowaxes® were melted on a water bath, the acetylsalicylic acid added, and mixed well. The mixture was allowed to cool to approximately 50° and then poured into suppository molds where solidification took place at room temperature.

On one day for three successive weeks eleven male volunteer subjects were administered ten grains of acetylsalicylic acid in suppository form in one of the three bases. On the fourth week, two five-grain aspirin tablets were given so that a comparison of oral to the rectal route could be established. At no time during this investigation was any other

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[1] A. S. A.® (Eli Lilly & Co.) suppositories purchased on the open market.

form of salicylate allowed the subjects. The suppositories were inserted in the morning, and two hours later 6 cc. of blood was obtained by venipuncture. This blood, oxalated, was centrifuged for five minutes at 2,000 r. p. m. to effect separation of the cells from the plasma.

Brodie's method (12) modified, was used for the determination of salicylic acid in plasma:

(*a*) Two cubic centimeters of plasma was pipetted into a separatory funnel in which five drops of 10% sodium hydroxide solution were added. (*b*) This mixture was shaken for five minutes. (*c*) By means of a pipet, 0.6 cc. of 6 *N* hydrochloric acid was introduced into the separatory funnel, followed by five minutes of vigorous shaking. (*d*) Thirty cubic centimeters of carbon tetrachloride was added, followed by shaking on an automatic device for five minutes to allow complete extraction of the salicylate. (*e*) The two immiscible layers were allowed to separate, after which the water layer was discarded. (*f*) To the nonaqueous layer 0.25 cc. of 1% ferric nitrate solution in 0.07 *N* nitric acid was added, followed by 10 cc. distilled water. (*g*) This mixture was shaken for five minutes and the nonaqueous layer subsequently discarded. (*h*) A portion of the aqueous layer was collected in a Klett tube and centrifuged for three minutes at 2,000 r. p. m. (*i*) The tube was placed in a Klett colorimeter from which the concentration of salicylic acid present, as indicated by the intensity of the blue color, was determined in milligrams per 100 cc. of plasma.

A standard graph (mg. per cent aspirin plasma levels) was prepared by treating plasma with known quantities of salicylate and using this for comparison of the unknown (see Fig. 1). Milligram per cent aspirin-plasma levels were read from the graph after obtaining respective readings from a Klett colorimeter. The results obtained were as follows:

(*a*) Absorption from oral tablets, 3.51 mg. % assigned the value of 100%. (*b*) Absorption from Carbowax® base, 3.27 mg. % or 93.1%. (*c*) Absorption from cocoa butter base, 2.30 mg. % or 65.5%. (*d*) Absorption from glycerinated gelatin base, 1.86 mg. % or 52.9%.

Thus it appears that when acetylsalicylic acid suppositories of the water-soluble Carbowax® base are administered, the rectal dosage given is equivalent to the oral, as a high degree of absorption through this vehicle is assured when employed rectally.

Of the results obtained from the eleven subjects, nine could be grouped and analyzed statistically, while the two remaining were discarded due to the inability of the subjects to retain the suppositories.

Following insertion of the suppositories, the test group concerned was questioned regarding irritation of the rectal mucosa. Any burning, soreness, and a very strong desire to defecate were taken as signs of irritation. It was shown that rectal irritation from acetylsalicylic acid suppositories of the cocoa butter and Carbowax® variety was virtually nonexistent, while those of glycerinated gelatin demonstrated a high incidence of prolonged burning and pain. Regarding glycerinated gelatin, the problem of maintaining the suppository in place for the prescribed length of time became a serious one, as each subject evidenced a very strong desire to expel this agent. It would appear that the incorporation of acetylsalicylic acid in a suppository consisting of a glycerinated gelatin base would be the least desirable from the point of patient comfort and tolerance.

A most important aspect of the problem was to determine the degree of stability of acetylsalicylic acid in the various types of bases used. After the aspirin was incorporated in the respective bases by the fusion process, several of each of the suppositories were placed in closed, airtight containers, and kept at room temperature for a six-month period. At no time were the suppositories refrigerated. Upon inspection, no color changes of the suppositories could be detected. No sublimation of crystals to the surface of the suppositories took place.

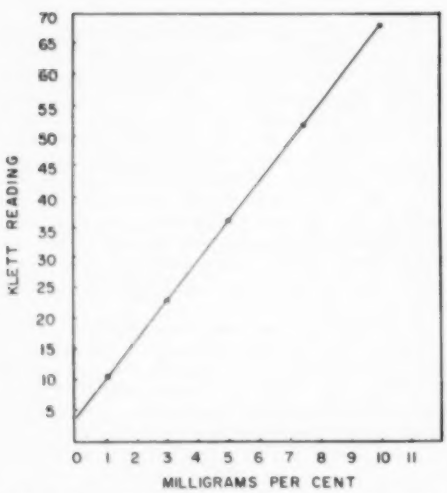

Fig. 1.—Milligrams per cent aspirin-plasma levels.

SUMMARY AND CONCLUSIONS

1. Plasma levels of acetylsalicylic acid administered rectally in three different types of suppository bases were compared to plasma levels of aspirin administered orally.

2. No statistical difference is evidenced in the degree of absorption of acetylsalicylic acid between a base of glycerinated gelatin and cocoa butter.

3. Statistical analysis reveals that no significant difference exists between the absorption of acetylsalicylic acid from the Carbowax® type base given rectally and acetylsalicylic acid administered orally.

4. The difference in the absorption of acetylsalicylic acid from the glycerinated gelatin and cocoa butter bases, as compared to the oral and absorption from the Carbowax® base, is statistically significant.

5. Irritation studies conducted reveal that little or no irritation results from administration

of acetylsalicylic acid suppositories of both the cocoa butter and Carbowax® types, while glycerinated gelatin causes a high incidence of irritation.

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The Physics of Tablet Compression*

V. Studies on Aspirin, Lactose, Lactose-Aspirin, and Sulfadiazine Tablets

By T. HIGUCHI, L. N. ELOWE †, and L. W. BUSSE

Results obtained from studies on the dependency on compressional force of various physical properties of compressed tablets are shown to be in good qualitative agreement with those found in an earlier investigation on sulfathiazole tablets. Such differences in behavior of several granulations as were found could be attributed mainly to the difference in relative hardness and cohesive properties of the substances employed.

THE INFLUENCE of maximal force used in compression on the physical properties of tablets prepared from a single sulfathiazole granulation was discussed in an earlier publication (1). Since substances commonly compressed into tablets vary in their physical and chemical properties and range from inorganic salts to complex organic molecules, the relations obtained in the case of the sulfathiazole studies may not necessarily hold in all tablet formulas. The purpose of the present work was to study the compressional behavior of several other formulations and determine whether or not the relations observed in the earlier investigation were generally valid.

The compounds for the present study were therefore selected to provide a representative sample, in so far as physical and chemical properties are concerned, of substances commonly compressed into tablets. This paper deals specifically with results obtained from compression of sulfadiazine, aspirin, lactose, and lactose-aspirin tablets.

As in the earlier study, the following physical characteristics and properties of tablets were determined as functions of the maximal force used during the compressional process: (*a*) specific surface area, (*b*) apparent and true densities, (*c*) hardness, and (*d*) disintegration time.

In the study of the disintegration time of sulfathiazole tablets (1), only one per cent dried cornstarch was used in all the tablets. In the present study, a more extensive investigation of

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The paper is based in part on a dissertation submitted to the Graduate School of the University of Wisconsin by L. N. Elowe in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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the disintegration rates of sulfadiazine tablets was undertaken by incorporating various proportions of dried cornstarch in different batches of the sulfadiazine granulation.

RESULTS AND DISCUSSION

In nearly all cases, the results obtained by these studies were in general qualitative agreement with those previously found in the sulfathiazole granulation (1). Such differences as were found could be attributed mainly to the difference in relative hardness and cohesive properties of the substances employed.

Specific Surface Area.—The observed influence of compressional force on the specific surface area of the tablets in the present study is qualitatively similar to that observed in the case of the sulfathiazole granulation. Initially, an increase in the maximal compressional force resulted in larger values for the specific surface area of the tablets. This relation continued until a certain force was reached after which further increments of force apparently caused the specific surface area to decrease. The value of the observed maximum specific surface area, and the force at which it appeared, varied with the structure of the substances compressed. The nature of the relation between specific surface area of the tablets and the optimal compressional force is shown in Figs. 1 and 2.

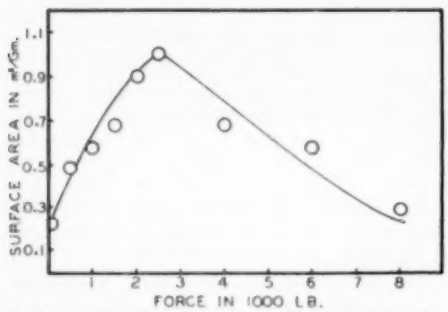

Fig. 1.—The effect of compressional force on the specific surface area of sulfadiazine tablets.

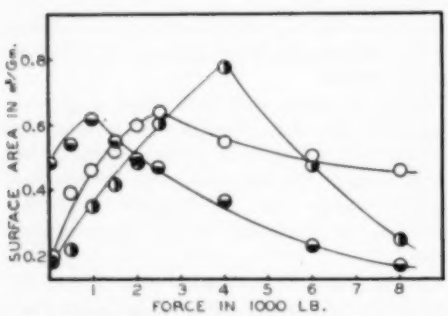

Fig. 2.—The effect of compressional force on the specific surface area of various tablets. ◒ aspirin, ◑ lactose, ○ lactose-aspirin.

An examination of these figures will show that sulfadiazine tablets resemble to a great extent the sulfathiazole tablets of the earlier study (1), both in the position of the maximum specific surface areas and in their values. This was expected in view of the closely related structures of the two substances.

Aspirin particles appear to be easily crushed as can be inferred from the rapid increase in their specific surface area which reached a peak at only 1,000 lb. force, with the 3/8-inch punch and die used. In the case of lactose, the force required to obtain the maximum specific surface area is 4,000 lb., indicating that lactose particles are more resistant to crushing.

Specific surface-area values between those of lactose and aspirin tablets were expected for the lactose-aspirin formula. In this case, the maximum specific surface area appeared at 2,500 lb., as expected, but the surface areas did not show values between those of lactose and aspirin tablets at every force level. A marked deviation in the force range of 6,000–8,000 lb. occurred in particular. In this range, the specific surface areas of the tablets made from the mixed granulation remained higher than those made from either constituent.

The relationship between the specific surface area of the different tablets and their porosity at various force levels is illustrated in Fig. 3. It can be seen that all the tablets exhibit maximum specific surface area at around 10% void space, even though the forces at which these maxima occur vary widely with the different formulations studied.

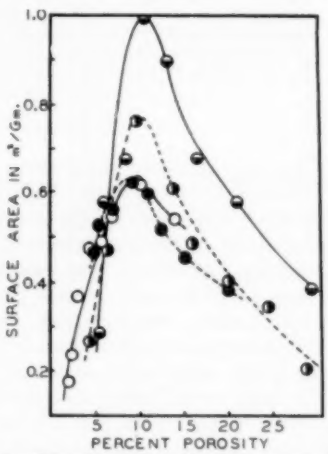

Fig. 3.—Porosity *vs.* specific surface area of various tablets. ◒ Sulfadiazine, ○ aspirin, ◑ lactose, ● lactose-aspirin.

Since it is necessary, in the method used for measuring surface areas, that nitrogen gas penetrate all capillary spaces within the tablet, it would be expected that with small void spaces penetration of the gas into the tablet might be impaired. At less than 10% void space, the surfaces of the tablets appear to prevent the free penetration of nitrogen gas into all void spaces.

An essentially similar relationship between specific surface area and compressional force was obtained in the case of the aspirin tablets made from precompressed granulation[1] and the tablets prepared from the original crystals used in the manufacture of the precompressed granulation. The maximum specific surface areas occurred for both formulations at 1,000 lb. and, at corresponding compressional force levels, the surface areas were of the same order of magnitude. The specific surface area of the crystals was about one-fourth that of the precompressed granulation. This indicates that there is an appreciable decrease in the particle size of the materials during the slugging process.

Porosity and Apparent Density.—Here again, the qualitative relationships of porosity and apparent density with maximal compressional force follow the same pattern as that observed in the case of sulfathiazole tablets (1). There appears to be a good correlation between porosity and molecular structure in the comparison of sulfadiazine with the sulfathiazole tablets. Figure 4 shows that sulfadiazine tablets have a porosity of 29.3% when compressed at a maximal force of 500 lb.; this decreases to 5.5% at 8,000 lb. The corresponding values for sulfathiazole tablets were 29.9 and 4.2%.

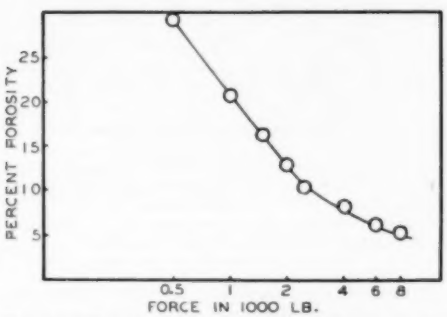

Fig. 4.—The effect of compressional force on the porosity of sulfadiazine tablets.

Figure 5 illustrates that aspirin tablets have porosities of 13.7 and 2% at 500 and 8,000 lb. force, respectively. The porosity of lactose tablets at these two forces are, respectively, 28.7 and 4.2%. Except for a small deviation in the force range of 6,000–8,000 lb., the porosity of lactose-aspirin tablets is of a magnitude between that of the individual lactose and aspirin tablets at corresponding force levels.

Since the apparent densities of tablets were determined for the purpose of the calculation of porosities, it was of interest to examine their relationship with the maximal compressional force. A direct linear relationship was found between apparent densities and the logarithm of the maximal force of compression, except at high-force levels where a deviation from this relation was obtained. This can be expected in view of the fact that with increased compressional force the apparent density of the tablets tends to approach the true density of the granulation as a limit.

[1] Precompressed granulation obtained from Dow Chemical Co., Midland, Mich.

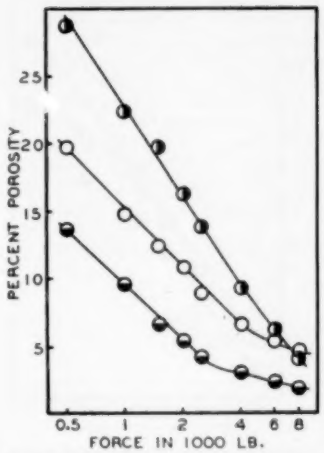

Fig. 5.—The effect of compressional force on the porosity of various tablets. ◒ aspirin, ◑ lactose, ○ lactose-aspirin.

Hardness.—A direct linear dependency of hardness of tablets on the logarithm of the maximal compressional force, like that in the study of the sulfathiazole granulation, was found for all present systems. A deviation occurred at high-force levels as in the relationship of apparent density of the tablets. This is illustrated in Figs. 6 and 7 which also show that lactose-aspirin tablets have hardness values between those of aspirin and lactose tablets separately.

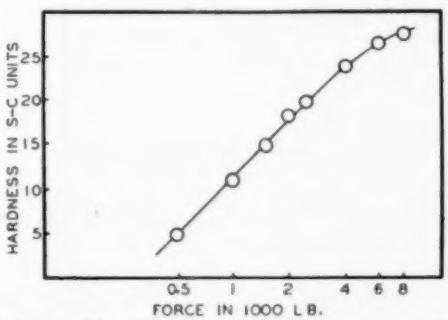

Fig. 6.—The effect of compressional force on the hardness of sulfadiazine tablets.

Since tablet hardness was determined with a Strong-Cobb hardness tester which measures resistance to breaking, the relations found are only valid for this property of the tablets. Further, as in the case of disintegration times, the figures plotted for the hardness of tablets represent the mean of five values obtained at each compressional force level.

Disintegration Time.—The disintegration times of lactose, aspirin, and lactose-aspirin tablets as a function of compressional force are presented in Fig. 8. All these tablets contained 10% dried cornstarch as a disintegrating agent. An examination

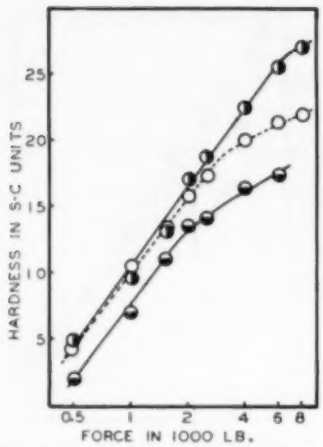

Fig. 7.—The effect of compressional force on the hardness of various tablets. ◒ aspirin, ◑ lactose, ○ lactose-aspirin.

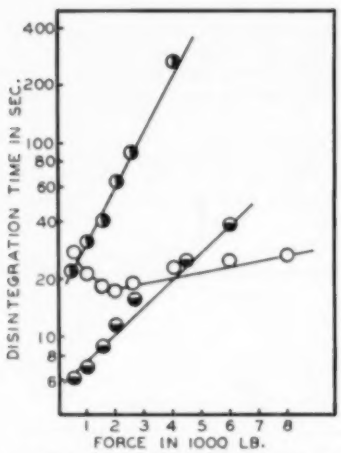

Fig. 8.—The effect of compressional force on the disintegration time of various tablets. ◒ aspirin, ◑ lactose, ○ lactose-aspirin.

of the figure shows that lactose-aspirin tablets have the most rapid rate of disintegration , the optimum force of 2,000 lb. Also, at higher force levels, the disintegration rate of the lactose-aspirin tablets is relatively fast when compared to that of the separate lactose and aspirin tablets. Further, the pronounced minimum which occurs in the curve for the lactose-aspirin tablets is absent in the other two cases.

The comparatively rapid rate of disintegration of lactose-aspirin tablets at high compressional force levels can perhaps be explained by the larger porosities retained allowing more rapid penetration of water, and further by the greater specific surface area of these tablets.

A more extensive investigation was carried out on the influence of the disintegrant concentration on the disintegration rate of sulfadiazine tablets. In this case, starch was added as disintegrant in four different proportions (15, 10, 5, and 1%) for comparative studies. The results are given in Fig. 9 which shows a minimum in the curve of the time-force relation for sulfadiazine tablets containing 15% dried cornstarch. This minimum is less pronounced in the case of the tablets with 10 and 5% dried cornstarch and is completely absent with those containing only 1% of the same disintegrant. It is also seen from Fig. 9 that the slope of the disintegration time-force curves increases with decreasing percentage of disintegrant.

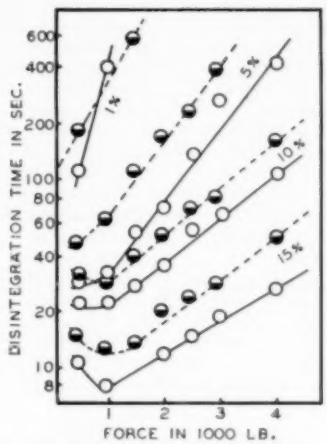

Fig. 9.—The effect of compressional force on the disintegration time of sulfadiazine tablets with various percentages of dried cornstarch. ○ modified B. P. method, ◒ modified U. S. P. method.

The above results are in general agreement with those observed by Berry and Ridout (2) who studied phenacetin tablets with 15% potato starch and phenacetin tablets with various proportions of alginic acid as disintegrating agents. These authors determined the disintegration times of the different tablets as a function of the ratio of the weight to height of the tablets which they called "compression ratio." It is obvious that this compression ratio is a measure of the maximal force applied during the compression of the tablets. They found that, up to an optimum point, the disintegration time decreased with increased compression ratio. After this point, however, increased compression ratio resulted in lower rates of disintegration. When various proportions of alginic acid were used, they found that the curves representing the compression ratio against disintegration time of the tablets had steeper slopes with decrease in the percentage of alginic acid added.

The reason for the occurrence of the minima in the curves representing disintegration time against maximal compressional force of certain tablets has been offered by Berry and Ridout (2). This explanation takes into account the swelling of starch grains and the porosity of the tablets. Under a very light compression, the grains can swell, but owing to large void spaces there is a certain lag period before

they begin to exert pressure on the surrounding granules. At the critical compression, as soon as the grains swell they exert such pressure on the surrounding granules that the tablets disintegrate rapidly. More time is required for the disintegration fluid to seep through the outer layers of tablets made under heavy compression and, as a result, slower disintegration rates are obtained for such tablets.

EXPERIMENTAL

Granulations for sulfadiazine and lactose tablets were prepared by adding 10% starch paste to the compounds until the masses were suitable for granulation. After granulating, drying was effected for four hours at 110° F. and then the dried granules were ground. The granules used for compression into tablets were those passing through a No. 20 but not through a No. 60 sieve.

Two series of aspirin tablets were studied. These were made from (*a*) Dow's commercial aspirin granules which are prepared by slugging and which contain 10% cornstarch, and (*b*) a mixture of aspirin crystals and 10% dried cornstarch.

Equal weights of lactose and aspirin crystals, incorporating 10% dried cornstarch, were the basis of the formulation for the lactose-aspirin tablets.

An arbitrary weight of 0.3950 Gm. of sulfadiazine granulation was taken for compression into tablets. Weights of the other formulations corresponding to the same true volume occupied by the sulfadiazine tablets were calculated. This was made possible by obtaining the true densities of all the formulations on a helium densitometer described earlier (3, 4). Table I shows the true densities and the weights compressed of each formula.

TABLE I.—TRUE DENSITIES AND WEIGHTS COMPRESSED INTO TABLETS OF DIFFERENT FORMULATIONS

Formulation	True Density, Gm./cc.	Wt. Compressed, Gm.
Sulfadiazine granules	1.554	0.3950
Lactose granules	1.552	0.3945
Lactose-aspirin crystals	1.485	0.3775
Aspirin (Dow's granules)	1.404	0.3570
Aspirin (from crystals)	1.383	0.3515

The tablets were compressed at eight varying force levels, ranging from 500–8,000 lb. per tablet, by means of a mechanical lever machine and a set of $^3/_8$-inch die and punches which have already been described (3).

Specific surface areas, apparent densities and hardness of the compressed tablets were determined by means of the B. E. T. low-temperature nitrogen adsorption apparatus, a specially designed mercury displacement tablet pycnometer, and a Strong-Cobb tablet hardness tester (1, 3, 4), respectively.

For disintegration time measurements, the method of the U. S. P. (5) was used with the substitution of a No. 8 for a No. 10 screen. A second disintegration method based on the B. P. procedure (6) was also used, employing a tube of 18 x $^5/_8$-inch effective measurements. When filled with water at 37° an empty space of 1 inch was left for bubble forming. The use of a constant temperature water bath was not found necessary in this method owing to the small temperature coefficient of tablet disintegration and to the very small fall in the temperature of the water during disintegration-time measurements of normal tablets (7).

Porosity was calculated from the following expression:

$$\text{Porosity} = \left(1 - \frac{\text{Apparent density}}{\text{True density}}\right) \times 100$$

For true density of the tablets, the true density of the granulation was used in the above expression.

SUMMARY AND CONCLUSIONS

1. The specific surface area of tablets increases with increased maximal compressional force to a maximum, and then decreases.

2. Apparent density varies directly, and porosity inversely, with the logarithm of the compressional force, up to a force level well above that used in ordinary compression of tablets.

3. Hardness varies directly with the logarithm of compressional force, leveling off at high forces. It also varies directly as the apparent density of tablets.

4. The logarithm of the disintegration time of tablets varies directly with compressional force. Higher rates of disintegration are produced by increasing the proportions of dried cornstarch added.

5. In some instances where a high percentage of disintegrating agent is present, faster rates of disintegration of the tablets are obtained as the maximal compressional force is increased. This continues up to an optimum compression after which the ordinary relationship of disintegration time with compressional force is manifested.

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A Study of the Adaptability of Isopropyl Myristate for Use as a Vehicle for Parenteral Injections*

By EDWARD L. PLATCOW and ELBERT VOSS†

Studies of isopropyl myristate show that it has a very low degree of irritability and exhibits no sensitizing propensities in animals following topical and parenteral administration. It is nontoxic to animals, and well tolerated following injections of moderate amounts. Its mixture with aluminum monostearate in 2 per cent solution of the latter compound retains effective therapeutic concentrations of penicillin (procaine penicillin) for periods up to forty-eight hours after intramuscular injection in rabbits. In experiments on ovariectomized rats it compared favorably with sesame oil as a repository vehicle for estrogens.

ISOPROPYL MYRISTATE is a clear, colorless, virtually odorless liquid (1). We have determined that it forms a thixotropic mixture with 10% beeswax but not with amounts of beeswax less than 10%. It is oil miscible, water immiscible, stable and not susceptible to rancidity; it is reported to have good solvent powers, a low order of sensitization when applied to the skin of humans, and a low toxicity when administered topically and orally to rabbits and rats, respectively (1). In recent years, isopropyl myristate because of its properties is being widely used as a substitute for vegetable oils in many cosmetic (2) and pharmaceutical preparations (3). Due to the physical similarity of isopropyl myristate to many of the vegetable oils now being used as repository vehicles, an investigation was made to determine if this compound might also be adapted to such use. Experiments were conducted to determine its toxicity, usefulness as a vehicle, and the extent of irritative effects. Animals were also tested to determine their sensitivity to this compound. The effects of isopropyl myristate on prolonging the therapeutic concentration of penicillin in the blood were studied in rabbits. Ovariectomized rats were employed in the studies of the adaptability of the compound for estrogenic repository administration.

EXPERIMENTAL

Acute Toxicity.—Due to the nontoxic effects of large amounts of isopropyl myristate administered orally to rats as reported elsewhere (1), it was felt that similar amounts should be administered intraperitoneally to mice to determine the acute toxicity by this route of administration. Male Swiss strain albino mice weighing 18 ± 1 Gm. were used. The animals were kept in an air-conditioned room during the test with the temperature maintained at 22°. Observations were made at three, twelve, thirty-six, and seventy-two hours.

Two animals were at first given 1 ml. (approximately 50 ml./Kg.) of isopropyl myristate intraperitoneally. There were no fatalities at the end of seventy-two hours. Two other animals were then given 2 ml. (approximately 100 ml./Kg.) in 1-ml. divided doses at an interval of four hours. At the end of seventy-two hours there were again no fatalities. It was impractical therefore to attempt an LD_{50} on isopropyl myristate.

Subacute Toxicity.—Tests were conducted to determine the effects of a short-term exposure to isopropyl myristate with reference to growth, weight of some vital organs, and histologic pathology.

A series of eight healthy young rats, unsexed, and weighing between 100 and 110 Gm. at the beginning of the test, was given 5 ml./Kg./day of isopropyl myristate over a period of twenty days by intraperitoneal injection. Five untreated rats were used as controls.

During the course of the experiment the rats were weighed once a week. The controls at the start weighed an average of 103 Gm. and the experimental animals weighed an average of 104 Gm. At the end of the three-week period the controls weighed an average of 186 Gm. and the surviving five rats of the experimental group averaged 194 Gm. An absence of growth depression was shown by the results of this test since there was no significant difference between the means of the two groups.

Three of the experimental rats were dead at the end of five days. Upon autopsy no gross pathology was noticed although part of the injected liquid remained unabsorbed in the peritoneal cavity. Death of these animals may have been due to the total volume of the liquid injected plus the mechanical trauma resulting from the daily injections rather than to any specific toxic effects.

The liver, heart, and kidneys were removed during autopsy from both the experimental and control groups. The weights of these organs were then tabulated and sections taken for microscopic study. There was no appreciable difference in organ weights between the experimental and control groups.

Histologic Pathology.—No histo-pathological changes were found upon examination of the following organs removed from rats exposed to the vehicle for twenty days by repeated intraperitoneal injec-

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tions: liver, kidneys, heart, intestine, spleen, adrenal glands, and gonads. Sections of muscle tissue revealed no pathology fourteen days after single injections of isopropyl myristate intragluteally but repeated daily injections for the same period caused some local irritation and necrosis which could have been due primarily to repeated wounding at the site of the injections. Signs of irritation were observed in rat muscle into which a mixture of 10% beeswax and isopropyl myristate was injected. The muscles were removed fourteen days after injection. Increased numbers of nuclei, mainly of fibroblast cells were observed in these sections. However, muscles of other rats into which 3 and 6% beeswax in the vehicle were injected were essentially normal in appearance at the end of fourteen days. Other muscle tissue from animals receiving 2% aluminum monostearate in the vehicle were also normal after a similar period of time.

Single intragluteal injections in rabbits of crystalline penicillin G sodium in a base of 10% beeswax and isopropyl myristate caused essentially some vascularized, congested masses of granulation tissue at the site of injection. Muscle tissue was removed fourteen days after these injections were made. After a corresponding period no observable lesions developed in sections taken after injecting crystalline penicillin G sodium in isopropyl myristate with 2% aluminum monostearate as a suspending agent. It seems most reasonable to conclude that the beeswax included in the first mixture was responsible for the frank irritation produced by the first mixture. Others have reported irritation in rabbits (4) and in humans (5) from injection of repository vehicles containing beeswax.

Sensitivity.—The procedure used in testing for sensitivity was essentially that of Landsteiner and Jacobs (6) in which the degree of sensitivity induced is determined by the extent of local inflammatory reaction produced by a final injection of isopropyl myristate two weeks after the last of a series of ten sensitizing injections given intracutaneously. Ten white male guinea pigs weighing 325–450 Gm. were employed in this test. The sensitizing and test readings were negative in all the animals. This indicates the absence of allergic or other types of sensitivity in the guinea pig.

Parenteral Irritation.—In order to test the irritation produced by isopropyl myristate after parenteral administration in the rabbit, the trypan blue test as devised by Hoppe, Alexander, and Miller (7) was employed. Five adult albino rabbits sexed at random weighing from 2 to 3 Kg. were used.

A volume of 0.3 ml. of isopropyl myristate was injected intracutaneously into two areas selected at random on the abdominal skin of the rabbit. A dose of 1 ml./Kg. of a 1% solution of trypan blue in normal saline solution was injected intraventricularly within ten to twenty minutes after completion of the intracutaneous wheals. The sites of the wheals were examined at one-half, one, and three-hour intervals after injection of the trypan blue solution. No appearance of color at the sites of the wheals at any of the time intervals in these animals after injection of the trypan blue solution indicated the absence of parenteral irritation.

Mucous Membrane Irritation.—A test for irritation to mucous membranes was carried out by the rabbit eye irritation method of Hoppe, Alexander, and Miller (7). Results of this test were graded according to the method of Draize, *et al.* (8). A maximum average irritation score of 2.8 was shown at one hour, a score of 1.2 at two hours, zero scores at four and eight hours. These scores indicate a mild irritation of only the conjunctiva at one and two hours after instillation, and no irritation to any part of the eye after four hours. According to Hoppe, *et al.* (7), there would be little likelihood of irritation from any parenterally injected compound which produced no more than a mild degree of irritation by this test.

Penicillin Blood Levels.—The following penicillin preparations were used in testing the effectiveness of isopropyl myristate as a repository vehicle: Penicillin G sodium with 2% aluminum monostearate in isopropyl myristate, penicillin G sodium in a mixture of 10% beeswax and isopropyl myristate, and procaine penicillin G with 2% aluminum monostearate in isopropyl myristate. In each case the product contained 100,000 units of the penicillin per ml. of preparation.

Table I gives the results of blood level studies in unsexed rabbits weighing from 2 to 3 Kg., using the sodium penicillin G and procaine penicillin G formulations mentioned above. The average blood level of six rabbits is given for each time interval. As a control, sodium penicillin G in saline is included. For these studies the rabbits were given 50,000 units/Kg. intragluteally. The blood samples were assayed by a filter-disk modification of the cup-plate method employing *Sarcina lutea* as the test organism (9). Pretreatment blood samples in all instances were negative.

As can be seen from Table I the procaine penicillin G product was the longest-acting preparation. It produced an effective therapeutic concentration for at least forty-eight hours.

Our results compare favorably with findings reported by Hobby, *et al.* (10), on blood levels of procaine penicillin G in sesame oil and peanut oil following intramuscular injections in rabbits. These authors reported that single injections of 30,-

TABLE I.—PENICILLIN BLOOD LEVELS

Preparation	Blood Level in Units per ml., hr. 1	2	8	8	12	24	48	72
A	12.53	4.28	0.55	0.06	...[a]	...	...	...
B	25.83	9.12	1.13	0.05	...[a]	...	...	...
C	5.21	2.80	0.65	0.18	0.05	...[a]	...	...
D	20.82	10.73	5.53	2.59	1.04	0.12	0.06	...[a]

A—Crystalline penicillin G sodium in normal saline.
B—Crystalline penicillin G sodium with 2% aluminum monostearate in isopropyl myristate.
C—Crystalline penicillin G sodium in a mixture of 10% beeswax and isopropyl myristate
D—Procaine penicillin G with 2% aluminum monostearate in isopropyl myristate.
[a] Less than 0.05 u./ml.

000 units per Kg. weight (our injections contained 50,000 units/Kg.) produced detectable blood levels lasting twenty-four to forty-eight hours. They report that the use of aqueous rather than oil solutions of procaine penicillin G gave less prolonged penicillin blood levels.

Estrogenic Activity.—Since many hormonal compounds especially the gonadal hormones used in therapeutics are administered in repository form, a study was made to determine the adaptability of isopropyl myristate for estrogenic repository use. The vaginal smear method, a modification of the Allen-Doisy test used in bioassays of estrogenic compounds (11), was employed in this test. Eight female rats were ovariectomized. Completeness of the operation was checked by vaginal smears for ten days postoperatively. A 0.05-mg. dose of α-estradiol was administered subcutaneously in 0.5 ml. of the vehicle. The α-estradiol was first dissolved in 0.05 ml. of acetone before adding the isopropyl myristate.

A first smear was taken three days after the injection and others at six- and nine-day intervals. A proestrus smear containing chiefly nucleated epithelial cells or nonnucleated cornified cells was taken as a criterion of a positive response. At the three-day interval a positive estrus smear was taken, and at six- and nine-day intervals negative anestrus smears, made up essentially of leucocytes and some nucleated epithelial cells, were obtained.

These results were the same as prevailed in controls given α-estradiol in sesame oil.

SUMMARY

1. Studies of isopropyl myristate show that it has a very low degree of irritability and exhibits no sensitizing propensities in rabbits and guinea pigs following topical and parenteral administration.

2. It is nontoxic to mice, but its lack of toxicity for rats seems questionable since three out of eight rats died after five daily intraperitoneal injections of 5 ml./Kg. wt.

3. Its mixture with aluminum monostearate in a 2 per cent solution of the latter compound retains effective therapeutic concentrations of penicillin (procaine penicillin) for periods up to forty-eight hours after intramuscular injection in rabbits.

4. In experiments on ovariectomized rats it compared favorably with sesame oil as a repository vehicle for estrogens.

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WHO MAKES IT?

The Registry of Rare Chemicals, Armour Research Foundation of the Illinois Institute of Technology, 35 West 33rd Street, Chicago 16, Ill., seeks information on sources of supply of the following chemicals:

Tangeretin
Thevetine
Murexine
Meroxyl
Crotonyl isothiocyanate
Ketoglutaric aminase
Cyclophorase
4-Ureido-5-Imidazole carboxylic acid
Nobiletin
Arecolidine
β-*o*-Cresyl glutaric acid
Mucinase
p-Iodophenyl isothiocyanate
Gentisic aldehyde
Diethylphosphine
1,2,4,5-Tetrahydroxybenzene
2,5,4′-Triethoxydiphenyl-1-diazonium chloride
cis-Propenyl bromide
2,4,2′,4′-Tetrahydroxydiphenyl
Anthracene-9,10-dicarboxylic acid

The Use of Some Imitation Flavors for Masking Distasteful Drugs*

IV. Variations in Concentrations of Flavoring Substances

By DURWARD NEAL ENTREKIN† and CHARLES H. BECKER‡

With quinine hydrochloride as the distasteful drug, an investigation was carried out to see if increasing the amount of such substances as salt, acid, and sugar over the normal quantities usually accepted would increase the disguising power of the syrups used. Doubling the flavor concentration, increasing the sugar content to approach that of simple syrup, adding salt to some flavored syrups and excess citric acid to others, and increasing the viscosity of the taste test solutions—all helped considerably in disguising the bitter taste of quinine hydrochloride. Both skimmed and homogenized milks were found to be better than water as the diluent for the taste-test solutions. Homogenized milk was better than skimmed milk. Cacao syrup U. S. P., was found to be the best-flavored syrup for masking the bitter taste of quinine. The other official syrups, cherry and raspberry, and the imitation flavored syrups, coconut, cream soda, butterscotch, and wild cherry, acid added, were found to be consistently high in disguising ability.

LANKFORD and Becker (1, 2) and then later Entrekin and Becker (3) evaluated the disguising powers of some imitation flavors actually prepared from known materials in the laboratory on ammonium chloride, quinine hydrochloride, and castor oil, respectively. Boothe and Kaufman (4) determined the masking effectiveness of ninety combinations of flavors, including synthetic materials, for quinine sulfate. Lathrop and Clark (5) used some imitation flavors in their attempts to disguise the bitter taste of the vitamin B complex. However, in all these cases, no studies were made varying the concentration of flavor or sugar, nor were studies carried out to see if added substances, such as salt and citric acid would enhance the disguising powers of these flavors to mask better the taste of a distasteful drug. It was, therefore, the purpose of this investigation to evaluate these factors, using quinine hydrochloride as the distasteful drug.

EXPERIMENTAL

The materials and formulas of imitation concentrates, preparation of syrups, taste test samples, and controls, as well as the method of evaluation of the vehicles used in this investigation were the same as described in a previous paper (1) with some modifications indicated below.

The concentrations of quinine used in this investigation were the same as those used by Purdum (6) and Lankford and Becker (2) except that the next higher concentration, number 15, was added. The normality of this concentration was 0.00086 and each 4-cc. taste-test sample contained 0.0014 Gm. of quinine hydrochloride.

The syrups of double-flavor strength were prepared by adding 3.34 cc. of the flavor concentrate to enough simple syrup to make 1,000 cc. In acidified imitation-flavored syrups where double acid content was used, 8.00 Gm. of citric acid was dissolved in enough flavored syrup to make 1,000 cc. In those imitation-flavored syrups where salt was added, 2.5 Gm. of sodium chloride was dissolved in enough of the flavored syrup to make 1,000 cc.

In the experiment to determine the effect of increasing the sweetness on masking ability, a known amount of quinine hydrochloride solution was mixed with one fluid ounce of simple syrup or flavored syrup, and then another fluid ounce of simple syrup and enough distilled water was added to make six fluid ounces. This doubled the sweetness of the vehicle. Vehicles with more than twice the sweetness were prepared by using suitable amounts of simple syrup. When a vehicle equivalent in sweetness to simple syrup was desired the alkaloidal salt solution was first mixed with simple syrup or flavored syrup, three fluid ounces of simple syrup, 102 mg. of soluble saccharin, and enough distilled water to make six fluid ounces were added.

To increase the viscosity of the vehicle 0.6 Gm. of CMC, HV, per six-fluid ounce drink, was added.

Skimmed milk was prepared by dispersing 8.75 Gm. of powdered skimmed milk in enough distilled water to make 100 cc. This dispersion or homogenized milk, wherever indicated, was used in place of distilled water to make the vehicle.

Fifteen persons constituted a taste-test panel throughout this investigation.

Results of Tables I–VI show that cacao syrup U. S. P., was found significantly better than any of the other syrups for disguising the bitter taste of quinine. However, all of the syrups were better than water or reconstituted skimmed milk as disguising agents. Comparing the significance of dif-

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TABLE I.—THE SIGNIFICANCE OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION-FLAVORED AND OFFICIAL SYRUPS OF DOUBLE-FLAVOR CONCENTRATION FOR QUININE HYDROCHLORIDE

Syrup	Mean Threshold in Water ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Syrup ± Standard Error, $m_1 \pm e_1$	Mean Disguising Potential ± Standard Error, $D \pm e$	Significance of Difference
Cacao syrup U. S. P.	3.20 ± 0.28	14.13 ± 0.27	10.93 ± 0.27	28.09
Imitation coconut syrup	3.80 ± 0.24	9.27 ± 0.15	5.47 ± 0.19	19.33
Imitation cream soda syrup	3.80 ± 0.24	9.00 ± 0.20	5.20 ± 0.26	16.67
Imitation raspberry syrup	3.80 ± 0.24	8.87 ± 0.19	5.07 ± 0.33	16.57
Imitation wild cherry syrup	3.80 ± 0.24	8.80 ± 0.17	5.00 ± 0.30	17.01
Imitation wild cherry syrup, acid added	3.00 ± 0.14	8.00 ± 0.21	5.00 ± 0.28	19.84
Imitation grape syrup, acid added	3.00 ± 0.14	7.93 ± 0.21	4.93 ± 0.21	19.56
Imitation raspberry syrup, acid added	3.00 ± 0.14	7.67 ± 0.19	4.67 ± 0.19	20.30
Imitation root beer syrup	3.67 ± 0.27	8.20 ± 0.24	4.53 ± 0.31	12.55
Imitation maple syrup	3.67 ± 0.27	8.00 ± 0.20	4.33 ± 0.27	12.89
Imitation butterscotch syrup	3.67 ± 0.27	7.93 ± 0.18	4.26 ± 0.27	13.15
Imitation grape syrup	3.67 ± 0.27	7.60 ± 0.16	3.93 ± 0.30	12.51
Simple syrup U. S. P.	3.80 ± 0.24	7.67 ± 0.11	3.87 ± 0.30	11.59

TABLE III.—THE SIGNIFICANCE OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION-FLAVORED AND OFFICIAL SYRUPS OF DOUBLE-SUGAR CONCENTRATION FOR QUININE HYDROCHLORIDE

Syrup	Mean Threshold in Water ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Syrup ± Standard Error, $m_1 \pm e_1$	Mean Disguising Potential ± Standard Error, $D \pm e$	Significance of Difference
Cacao syrup U. S. P.	3.20 ± 0.28	14.20 ± 0.13	11.47 ± 0.28	36.35
Raspberry syrup U. S. P.	3.20 ± 0.28	10.20 ± 0.26	7.00 ± 0.35	18.32
Cherry syrup U. S. P.	3.20 ± 0.28	9.73 ± 0.18	6.53 ± 0.29	19.61
Imitation butterscotch syrup	3.13 ± 0.24	9.00 ± 0.26	5.87 ± 0.24	16.63
Imitation cream soda syrup	3.13 ± 0.24	8.77 ± 0.24	5.74 ± 0.30	16.93
Imitation wild cherry syrup, acid added	3.13 ± 0.24	8.73 ± 0.23	5.60 ± 0.23	16.87
Imitation maple syrup	3.13 ± 0.13	8.67 ± 0.27	5.54 ± 0.31	18.47
Imitation coconut syrup	3.20 ± 0.17	8.67 ± 0.21	5.47 ± 0.24	20.26
Simple syrup U. S. P.	3.13 ± 0.13	8.53 ± 0.29	5.40 ± 0.29	16.98
Imitation root beer syrup	3.13 ± 0.13	8.53 ± 0.26	5.40 ± 0.27	18.56
Imitation raspberry syrup, acid added	3.13 ± 0.24	8.47 ± 0.22	5.34 ± 0.27	16.38
Imitation grape syrup	3.13 ± 0.13	8.40 ± 0.29	5.27 ± 0.30	16.57
Imitation wild cherry syrup	3.20 ± 0.17	8.40 ± 0.25	5.20 ± 0.31	17.22
Imitation raspberry syrup	3.20 ± 0.17	8.27 ± 0.25	5.07 ± 0.25	16.79
Imitation grape syrup, acid added	3.13 ± 0.24	8.13 ± 0.16	5.00 ± 0.29	17.36

TABLE V.—THE SIGNIFICANCE OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION-FLAVORED AND OFFICIAL SYRUPS IN SKIMMED MILK FOR QUININE HYDROCHLORIDE

Syrup	Mean Threshold in Skimmed Milk ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Syrup ± Standard Error, $m_1 \pm e_1$	Mean Disguising Potential ± Standard Error, $D \pm e$	Significance of Difference
Cacao syrup U. S. P.	4.93 ± 0.28	14.80 ± 0.11	9.87 ± 0.24	32.79
Imitation cream soda syrup	4.80 ± 0.26	10.00 ± 0.22	5.20 ± 0.22	15.25
Imitation grape syrup	4.80 ± 0.26	10.00 ± 0.22	5.20 ± 0.33	15.25
Imitation raspberry syrup	4.80 ± 0.26	10.00 ± 0.22	5.20 ± 0.30	15.25
Imitation coconut syrup	4.80 ± 0.26	9.80 ± 0.26	5.00 ± 0.28	13.62
Imitation wild cherry syrup	4.80 ± 0.26	9.73 ± 0.23	4.93 ± 0.32	14.25
Imitation maple syrup	5.13 ± 0.24	10.00 ± 0.28	4.87 ± 0.28	13.20
Imitation root beer syrup	5.13 ± 0.24	9.87 ± 0.24	4.73 ± 0.29	13.95
Imitation raspberry syrup, acid added	5.13 ± 0.24	9.87 ± 0.29	4.73 ± 0.33	12.55
Raspberry syrup U. S. P.	4.93 ± 0.28	9.67 ± 0.29	4.73 ± 0.30	11.77
Cherry syrup U. S. P.	4.93 ± 0.28	9.40 ± 0.25	4.47 ± 0.32	11.92
Imitation butterscotch syrup	5.13 ± 0.24	9.60 ± 0.29	4.47 ± 0.27	11.86
Simple syrup U. S. P.	5.13 ± 0.24	9.20 ± 0.24	4.07 ± 0.30	10.80
Imitation grape syrup, acid added	4.93 ± 0.28	8.93 ± 0.23	4.00 ± 0.26	11.05
Imitation wild cherry syrup, acid added	4.93 ± 0.28	8.93 ± 0.25	4.00 ± 0.28	10.67

TABLE II.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF IMITATION-FLAVORED AND OFFICIAL SYRUPS OF DOUBLE-FLAVOR CONCENTRATION IN TABLE I FOR QUININE HYDROCHLORIDE

	Syrup											
Cacao[a]	Coconut[b]	Cream Soda[b]	Raspberry[b]	Wild Cherry[b]	Wild Cherry[b, c]	Grape[b, c]	Raspberry[b, c]	Root Beer[b]	Maple[b]	Butterscotch[b]	Grape[b]	Simple[a]
16.56	...	...	...	...	...	...	...	...	...	...	...	...
15.28	0.84	...	...	...	...	...	...	...	...	...	...	...
13.72	1.05	0.31	...	...	...	...	...	...	...	...	...	...
14.68	1.32	0.50	0.16	...	...	...	...	...	...	...	...	...
15.24	1.39	0.52	0.16	0.00	...	...	...	...	...	...	...	...
17.54	1.91	0.81	0.36	0.19	0.20	...	...	...	...	...	...	...
18.97	2.97	1.64	1.05	0.93	0.97	0.92	...	...	...	...	...	...
15.57	2.59	1.65	1.19	1.09	1.12	1.07	0.39	...	...	...	...	...
17.28	3.45	2.32	1.73	1.66	1.71	1.75	1.03	0.49	...	...	...	...
17.46	3.67	2.51	1.90	1.83	1.89	1.96	1.24	0.66	0.18	...	...	...
17.33	4.34	3.20	2.33	2.52	2.61	2.73	2.08	1.39	0.99	0.82	...	...
17.48	4.51	3.35	2.69	2.67	2.76	2.90	2.25	1.53	1.14	0.97	0.14	...

[a] U. S. P.
[b] Imitation.
[c] Acid added.

TABLE IV.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF IMITATION-FLAVORED AND OFFICIAL SYRUPS OF DOUBLE-SUGAR CONCENTRATION IN TABLE III FOR QUININE HYDROCHLORIDE

	Syrup													
Cacao[a]	Raspberry[a]	Cherry[a]	Butterscotch[b]	Cream Soda[b]	Wild Cherry[b, c]	Maple[b]	Coconut[b]	Simple[a]	Root Beer[b]	Raspberry[b, c]	Grape[b]	Wild Cherry[b]	Raspberry[b]	Grape[b, c]
9.98	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.23	1.03	...	...	...	...	...	...	...	...	...	...	...	...	...
15.18	2.66	1.75	...	...	...	...	...	...	...	...	...	...	...	...
13.98	2.73	1.89	0.34	...	...	...	...	...	...	...	...	...	...	...
16.22	3.35	2.51	0.81	0.37	...	...	...	...	...	...	...	...	...	...
14.19	3.12	2.33	0.84	0.46	0.16	...	...	...	...	...	...	...	...	...
16.26	3.61	2.81	1.18	0.70	0.39	0.18	...	...	...	...	...	...	...	...
15.02	3.57	2.76	1.25	0.82	0.54	0.33	0.19	...	...	...	...	...	...	...
15.60	3.62	2.85	1.30	0.84	0.56	0.34	0.19	0.00	...	...	...	...	...	...
15.76	3.76	3.01	1.46	0.99	0.73	0.49	0.36	0.15	0.16	...	...	...	...	...
15.12	3.74	3.02	1.56	1.11	0.87	0.63	0.52	0.31	0.32	0.17	...	...	...	...
15.00	3.85	3.14	1.71	1.25	1.04	0.78	0.69	0.47	0.49	0.34	0.16	...	...	...
17.02	4.49	3.81	2.31	1.71	1.56	1.18	1.16	0.86	0.90	0.74	0.51	0.33	...	...
16.02	4.40	3.73	2.31	1.77	1.62	1.27	1.25	0.99	1.01	0.86	0.65	0.47	0.18	...

[a] U. S. P.
[b] Imitation.
[c] Acid added.

TABLE VI.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF IMITATION-FLAVORED AND OFFICIAL SYRUPS IN SKIMMED MILK IN TABLE V FOR QUININE HYDROCHLORIDE

	Cacao[a]	Cream Soda[b]	Grape[b]	Raspberry[b]	Coconut[b]	Wild Cherry[b]	Maple[b]	Syrup Root Beer[b]	Raspberry[b, c]	Raspberry[a]	Cherry[a]	Butterscotch[b]	Simple[a]	Grape[b, c]	Wild Cherry[b, c]
	14.32	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	11.47	0.00	...	...	...	...	...	...	...	...	...	...	...	...	...
	12.13	0.00	0.00	...	...	...	...	...	...	...	...	...	...	...	...
	13.20	0.56	0.46	0.49	...	...	...	...	...	...	...	...	...	...	...
	12.35	0.69	0.59	0.62	0.16	...	...	...	...	...	...	...	...	...	...
	13.55	0.93	0.76	0.80	0.33	0.14	...	...	...	...	...	...	...	...	...
	13.63	1.29	1.07	1.27	0.67	0.46	0.35	...	...	...	...	...	...	...	...
	12.63	1.19	1.01	1.05	0.62	0.44	0.32	0.00	...	...	...	...	...	...	...
	13.35	1.27	1.05	1.11	0.66	0.46	0.34	0.00	0.03	...	...	...	...	...	...
	13.50	1.88	1.59	1.67	1.25	1.02	0.94	0.60	0.57	0.59	...	...	...	...	...
	15.00	2.10	1.71	1.81	1.36	1.10	1.03	0.66	0.61	0.64	0.00	...	...	...	...
	15.06	3.04	2.53	2.66	2.27	1.95	1.95	1.58	1.48	1.56	0.91	0.99	...	...	...
	16.58	3.32	2.86	3.02	2.62	2.26	2.28	1.87	1.74	1.84	1.14	1.26	0.18	...	...
	15.91	3.37	2.78	2.93	2.52	2.19	2.20	1.82	1.69	1.78	1.11	1.21	0.17	0.18	...

[a] U. S. P.
[b] Imitation.
[c] Acid added.

ferences of Tables I, III, and V with those of a previous study (2) it may be concluded that doubling the flavor concentration and doubling the sugar concentration definitely increased disguising power and that reconstituted skimmed milk was better than water.

Although statistical data are not shown, experiments were also carried out in this investigation to determine the significance of the mean disguising potentials of some imitation and official syrups with the following; quadruple sugar concentration, quadruple sugar concentration and saccharin, sodium chloride, double citric acid concentration, carboxymethylcellulose, and homogenized milk. The data of these experiments were used to determine which syrups varied significantly. Only the fruit-flavored syrups, namely, cherry and raspberry syrups U. S. P. and imitation wild cherry, grape, and raspberry syrups were used to study the effects of double citric acid concentration on the vehicle. In the taste tests where sodium chloride was added, the fruit-flavored syrups were omitted. The results of these tests showed that, in general, cacao syrup U. S. P., was found significantly better than any of the other syrups for disguising the bitter taste of quinine. The other official syrups, cherry and raspberry, usually occurred rather high in the order of disguising ability. Of the imitation flavored syrups coconut, cream soda, butterscotch, and wild cherry, acid added, were found to be best.

The above experiments also brought out the following conclusions: as the sweetness of the taste-test drink approached that of simple syrup, the disguising power also increased in direct proportion; the addition of salt enhanced the disguising power of all syrups tried, but it gave more effectiveness to cacao syrup U. S. P. than any of the others; the addition of citric acid beyond the normal quantity generally used in fruit syrups to give tartness assisted in producing more disguisibility, although many subjects reported difficulty in distinguishing between tartness of the acid and bitterness of the drug; increasing viscosity of the taste-test drinks increased the disguising power of all the flavored syrups, although many subjects commented that this seemed to decrease the sweetness of the vehicle; and homogenized milk was better than water as a vehicle and a little better than reconstituted skimmed milk.

SUMMARY

1. Using quinine hydrochloride as the distasteful drug, an investigation was carried out to see if increasing the amount of such substances as salt, acid, and sugar over the normal quantities usually accepted would increase the disguising power of flavored syrups. It was found that these did increase the disguising power of all syrups used.

2. Doubling the flavor concentration helped considerably in disguising the bitter taste of quinine hydrochloride.

3. Results showed that as the sweetness of the taste-test drink approached that of simple

syrup, the disguising power also increased in direct proportion.

4. The addition of salt to some flavored syrups, an excess amount of citric acid to the fruit-flavored syrups, and increasing the viscosity of the taste-test solutions—all increased the disguising power of the syrups in which they were used.

5. Both skimmed and homogenized milks were found to be better than water as the diluent for the taste-test solutions. Homogenized milk was better than skimmed milk.

6. Cacao syrup U. S. P., was found to be the best-flavored syrup for masking the bitter taste of quinine hydrochloride. The other official syrups, cherry and raspberry, usually occurred rather high in the order of disguising ability. Of the imitation flavored syrups, coconut, cream soda, butterscotch, and wild cherry, acid added, were found to be best.

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A Note on the Infrared Spectra of Diastereoisomers*

By R. A. CUTLER, C. M. MARTINI, and F. C. NACHOD

IT HAS BEEN postulated that the fingerprint regions of the infrared absorption spectra of diastereoisomers should show marked differences. Furthermore, it has been assumed that interference with hydrogen bonding effects should be more pronounced in one of the isomeric forms due to the spatial arrangement. Differences in spectral bands have been observed by Carter, *et al.* (1), while Bergmann and co-workers report no or only slight differences in the spectra of diastereoisomers (2).

From previous investigations (3) a series of diastereoisomeric pairs, related to threonine and allothreonine were available which were employed in an effort to solve the above-mentioned contradiction. The infrared spectra were determined as mulls owing to the poor solubility of the compounds in suitable solvents. The curves of the parent amino acid pair and of the 2-amino-1-(4-methylmercaptophenyl)-1,3-propanediol pair as a representative member of the studied series are shown in Figs. 1

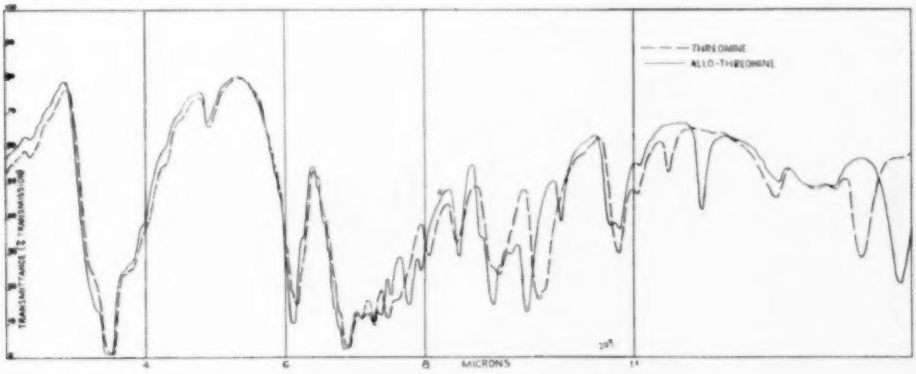

Fig. 1.—Infrared spectra of threonine and allothreonine.

* Received March 16, 1954, from the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

TABLE I.—INFRARED ABSORPTION MAXIMA OF 6 PAIRS OF *dl*-THREO AND *dl*-ERYTHRO ISOMERS

2-Dichloracetamido-1-(4-Methylsulfonylphenyl)-1,3-Propanediol		2-Acetamido-1-(4-Methylsulfonylphenyl)-1,3-Propanediol		2-Amino-1-(4-Methylsulfonylphenyl)-1,3-Propanediol		2-Amino-1-(4-Methylmercaptophenyl)-1,3-Propanediol		2-Dichloroacetamido-1-(4-Methylmercaptophenyl)-1,3-Propanediol		2-Acetamido-1-(4-Methylmercaptophenyl)-1,3-Propanediol	
dl-Threo, λμ	*dl*-Erythro, λμ	*dl*-Threo, λμ	*dl*-Erythro, λμ	*dl*-Threo, λμ	*dl*-Erythro, λμ	*dl*-Threo, λμ	*dl*-Erythro, λμ	*dl*-Threo, λμ	*dl*-Erythro, λμ	*dl*-Threo, λμ	*dl*-Erythro, λμ
2.89	2.89		2.93		2.90		3.00	3.00			2.94
2.96	3.00	3.04		3.02	2.98		3.05	3.13	3.13		3.00
3.11	3.14	3.07	3.10		3.04	3.18			3.28	3.09	3.09
								5.92			
5.90	5.95	6.08	6.08					5.96	5.99	6.07	6.15
6.27	6.27	6.24	6.27	6.25	6.20	6.28		6.08			
6.41	6.40	6.51	6.44	6.32	6.23	6.34	6.33	6.24	6.32	6.25	6.25
							6.40	6.36			
								6.57		6.54	6.39
								6.70	6.70	6.70	6.72
6.85	6.87	6.86	6.87	6.84	6.84	6.87	6.87	6.85	6.85	6.87	6.85
7.12		7.14		7.10	7.13	7.17	7.11	7.10	7.10	7.15	6.95
7.27	7.28	7.27	7.28	7.26	7.26	7.26	7.27	7.27	7.27	7.30	7.29
7.51				7.33		7.45		7.42	7.38	7.52	
7.60	7.58	7.54	7.55	7.55	7.51		7.56	7.54	7.61	7.56	
7.73		7.68	7.72	7.60		7.75	7.72	7.66	7.74	7.74	7.65
7.83	7.81			7.81	7.79			7.80	7.87	7.80	
		7.91	7.96	7.94		7.90		7.97	7.95	7.97	
8.05	8.32	8.31	8.29	8.14	8.01	8.04	8.08	8.23	8.25	8.31	8.00
8.13	8.44		8.50		8.49	8.33	8.30	8.32	8.40	8.40	8.44
8.53	8.53	8.66	8.77	8.70	8.80	8.45	8.44	8.47		8.46	8.51
8.75	8.78	8.73	8.96	8.92		8.70	8.57				8.89
						8.94			8.90	8.94	8.95
9.03	9.00			9.01		9.01	9.01	9.09	9.16	9.17	9.19
	9.24	9.23	9.22	9.15	9.24	9.14	9.19	9.20	9.27	9.27	
9.36	9.36	9.31	9.31	9.23	9.40	9.24		9.37	9.40	9.31	9.37
9.71	9.57		9.54	9.54	9.47	9.62	9.49	9.50	9.77	9.70	9.60
	9.88	9.66		9.69	9.56	9.72	9.63	9.73	9.80	9.86	
	9.97	9.85	9.88		9.87	9.92	9.87	9.98		9.99	9.90
		10.13			10.05	10.15	10.18	10.22		10.16	
10.36	10.37	10.37	10.37	10.43	10.38		10.32	10.39	10.40	10.25	10.29
10.46		10.50				10.70	10.62	10.50		10.35	
	10.85		10.89		10.85	10.98	10.83	10.67			10.93
11.10	11.22		11.30	11.38			11.30	11.10	11.10		
11.75	11.66	11.72	11.60		11.57		11.40	11.29		11.38	11.29
11.93					11.80	11.94		11.93	11.97	11.94	11.74
12.32	12.20	12.37	12.07	12.01		12.36	12.23	12.26	12.13		12.03
			12.98	12.90	12.84				12.36	12.55	12.29
13.07	13.05	13.20							13.10		13.07
13.48	13.67	13.73	13.61	13.77	13.75						13.65
13.90	13.98	13.90	13.92			13.90	13.83	13.88	13.89	13.74	13.75
14.27	14.39	14.50	14.40	14.32	14.33			14.05		14.27	14.49
14.65	14.76	15.12	14.98					14.90		15.10	14.94

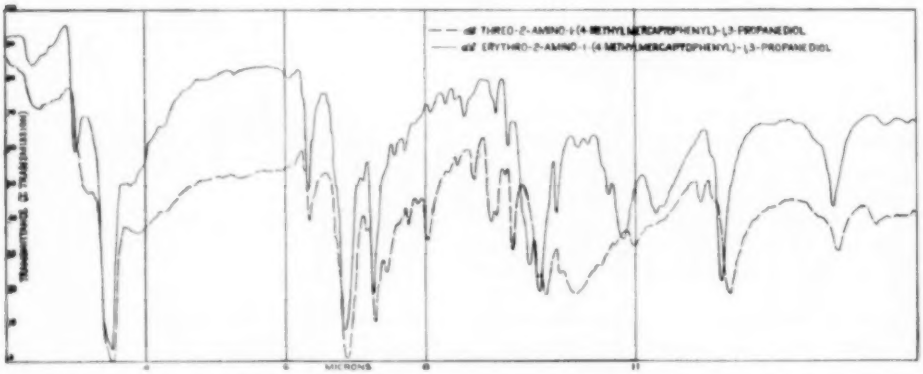

Fig. 2—Infrared spectra of *dl*-threo-2-amino-1-(4-methylmercaptophenyl)-1,3-propanediol and *dl*-erythro-2-amino-1-(4-methylmercaptophenyl)-1,3-propanediol.

and 2, respectively. While marked differences are apparent, a careful tabulation of the bands, given in Table I indicates that no clearcut correlation or structure assignment is possible, at least not in the present series. However, an assignment of α or β configuration in a series of sugar anomers (4) has been reported if the change from molecule to molecule is very limited.

Spectra of another limited series, the substituted β-phenylserines have just been reported (5). Here it is particularly interesting to note that the *erythro*-structure was assigned to compounds showing a band at 11.95μ. This is at variance with the findings presented in Table I, where in four out of six cases a band appearing at 11.93 or 11.94μ, respectively, is associated with the *threo*-configuration.

We would conclude that structure assignment in series of complex molecules, especially where the study of homogeneous solutions is not possible, is perhaps not free from ambiguity.

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A Note on the Phytochemical Study of the Root of *Cnidoscolus Texanus* (Muell. Arg.) Small*

By SUE H. ROUSE† and RALPH BIENFANG

The root of *C. texanus*, heretofore not examined chemically, has been found to be high in carbohydrate content with alkaloids and glycosides absent, or present only in negligible quantities. Other interesting facts observed in this investigation indicate that the root system as well as other parts of the plant might be worthy of further investigation.

Cnidoscolus texanus (Muell. Arg.) Small (*Jatropha texana*[1]), Family Euphorbiaceae, is a weed which grows in abundance throughout Oklahoma, Arkansas, and Texas (1). It is known locally as "Bull Nettle" and "Stinging Nettle." These are common names also applied to the closely related coastal plains species *Jatropha stimulosa* Michx. (2), (*J. urens* L.) (3), a scientific name which has been applied erroneously to the Oklahoma, Arkansas, and Texas plant. A survey of the literature concerning *C. texanus* reveals little information, and no chemical studies of the root of the weed have been reported.

* Received February 12, 1954, from the Research Laboratories, University of Oklahoma, College of Pharmacy, Norman, Okla.
† Present address: College of Pharmacy, University of Houston, Houston, Tex.
[1] According to Dr. George Goodman, Professor, Plant Science Department, University of Oklahoma, personal interview, March, 1949, this is the only synonym for the plant under investigation.

EXPERIMENTAL

The root of *C. texanus* is very succulent and exudes a milky juice when wounded. It may measure six feet in length and be 3–3$^1/_2$ inches in diameter. The root used in the investigation was obtained in May and weighed 25–30 lb. A moisture determination of the fresh root revealed 83% water.

The fresh root was cut in several places and some of the milky juice was collected. It was added to water and formed a latex-like mass when rolled between the fingers.

The root was air dried for six months, sawed into thin slices, then ground in a Wiley mill. The ground substance was a light, bulky mass of white powder and sawdust-like material, all of which passed through a No. 20 sieve.

Preliminary extraction procedures, using solvents in the order listed by Dragendorff, showed that the greatest yield of extractives would be obtained with alcohol and water. Since the white, powdered substance in the ground root was suspected to be starch, it was removed from a weighed sample to prevent its interference with other carbohydrate tests. This was accomplished by macerating the sample with cold water and washing the residue on a No. 60 sieve. The washings were found to contain an insoluble material and were saved for further treatment.

The washed sample of the ground root was partially dried and extracted successively with alcohol

and water. Standard carbohydrate tests were used on these percolates and they were found to contain glucose and fructose.

The insoluble, white substance contained in the aqueous liquid was further purified by repeated washings with cold water, filtered, and dried. A positive starch test was obtained with Iodine T. S. and microscopic examination of the dried granules showed that they were similar in size and appearance to those of cornstarch. This substance had no reducing properties. A sample was hydrolyzed and standard carbohydrate tests revealed that glucose was the only product formed.

None of the solutions under observation gave positive tests for gums, higher glycosides, or sucrose. The alcoholic solution gave negative tests for alkaloids.

Total carbohydrates, determined quantitatively by calculating on the basis of invert sugar by the method of Sachsse (4), were found to amount to 34%. A quantitative determination of the starch content of the ground root gave an average of 22%, leaving 12% of the carbohydrates to be principally glucose and fructose.

Samples of the dried root were submitted to further analysis and the average results were:

Moisture, %	11.14
Ash, %	8.11
Acid Insoluble Ash, %	2.37
Crude Fiber, %	26.12

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Salicylamide II.*

A Note on the Effect of Salicylamide on the Circulating Eosinophils and Ascorbic Acid of the Rat

By JAMES M. CRAMPTON† and ELBERT VOSS‡

Salicylamide was found to produce a significant decrease in eosinophils in four hours and a more marked decrease in six hours. Salicylamide also produced a significant decrease in ascorbic acid content of the adrenal glands. These effects are considered indicative of increased activity of the adrenal cortex.

IN RECENT YEARS it has been suggested that the salicylates have in addition to their analgetic action an effect on the output of adrenocortical hormones. Evidence linking salicylates with adrenocortical activity has been presented by numerous investigators. Keleman and Majoros (1), Van Cauwenberge and his associates (2–4) and Hetzel and Hine (5) report adrenocortical stimulus by large doses of salicylates as shown by eosinopenic response, decrease in adrenal cholesterol and ascorbic acid, and increased urinary excretion of steroids. Bach, *et al.* (6), reported similarity in action of salicylate and ACTH. The work of Calesnick and Beutner (7) and Schuman and Finestone (8) suggest that the hyaluronidase inhibiting action of salicylate may be due to its adrenocortical stimulating effect. Meade and Smith (9, 10) disagreed with the conclusion of Hetzel and Hine, Van Cauwenberge, and others, and reported failure to obtain eosinopenia in humans after salicylates and differences in action of salicylate and ACTH on glycosuria in rats. It seems however, that the preponderance of evidence supports the belief that salicylates have a stimulating effect on the adrenal cortex exerted through the pituitary. That salicylamide shares the pituitary-adrenocortical stimulating action of salicylates is suggested by the report of Cronheim (11) that salicylamide reduced the ascorbic acid content of the rat adrenal glands. Since salicylamide is apparently less toxic than salicylate and produces fewer side effects it might possibly prove to be a valuable substitute for the salicylates in those diseases such as rheumatic fever which require very large doses. Its effects on the pituitary-adrenal system are therefore significant.

* Received October 30, 1953, from the College of Pharmacy, University of Florida, Gainesville.
Abstract from a dissertation presented to the Graduate School of the University of Florida by James M. Crampton in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Pharmacology.
† Fellow of the American Foundation for Pharmaceutical Education. Present Address: Xavier University, Department of Pharmacology, New Orleans 18, La.
‡ Head Professor, Pharmacognosy and Pharmacology, University of Florida, College of Pharmacy.

EXPERIMENTAL

Eosinopenic Response.—The eosinopenic response to ACTH has been proposed by Thorn, *et al.* (12), as a test of adrenocortical function. A 50% reduction in the number of circulating eosinophils is said to indicate a functioning adrenal cortex. Using the method recommended by Best, *et al.* (13), we studied the effect of salicylamide on circulating eosinophils in albino rats.

Control.—Of ten rats given water by stomach tube, only one gave a positive Thorn test. One showed an increase in eosinophils of 400%. In eight of the ten cases the zero and four hour counts fell within the 95% limits of error intrinsic in the counting method [Best, *et al.* (13)]. The average reduction was 12%. All of five rats given ACTH gave a positive Thorn test. The average reduction was 82%.

Response to Salicylamide.—Twenty rats were given 100 mg. of salicylamide per kilogram of body weight. The drug was administered in suspension by stomach tube. Eosinophil counts four and six hours after administration are compared in Table I with counts made immediately after administration.

Ascorbic Acid Depletion Tests.—Sayers and coworkers (14, 15) have shown that adrenal function is marked by a decrease in the ascorbic acid content of the adrenal glands. This decrease is produced by ACTH and is the basis of assay of that drug. Cronheim (11) reports that salicylamide and many salicylate derivatives also produce this response. His work is extended somewhat in the following experiment, in that oral doses in several strengths are used.

Effects of Salicylamide.—Twenty-five rats were treated with salicylamide administered by stomach tube. Doses given were 100, 200, and 400 mg./Kg. Animals were sacrificed two, four, and six hours after administration and the adrenals removed and assayed by the method of Roe and Kuether (16). The results are given in Table II and compared with a control group. It may be seen from these data, that the ascorbic acid content falls sharply after administration of salicylamide and then begins to rise slowly. Moreover, the degree of ascorbic acid depletion is clearly related to the dose administered.

SUMMARY

1. The eosinopenic response and the ascorbic acid depletion of adrenals indicate hyperactivity of the adrenal cortex following salicylamide administration.

2. We found the ascorbic acid of the rat adrenals to be as significantly reduced after oral administration of salicylamide as Cronheim reports from subcutaneous injection.

TABLE I.—EOSINOPHIL RESPONSE TO SALICYLAMIDE FOUR-HOUR TEST

Rat	Eosinophils/cu. mm. 0 Hr.	4 Hr.	Reduction, %	Rat	Eosinophils/cu. mm. 0 Hr.	6 Hr.	Reduction, %
1	455	278	31	11	322	116	64[a]
2	311	178	43	12	621	75	87[a]
3	77	61	21	13	218	44	78[a]
4	111	210	−91	14	539	126	77[a]
5	472	94	80[a]	15	349	249	29
6	311	77	75[a]	16	204	122	40
7	120	56	53[a]	17	233	61	74[a]
8	183	31	83[a]	18	459	84	82[a]
9	100	75	25	19	165	38	77[a]
10	50	22	56[a]	20	117	44	62[a]
Total	2190	1082	Av. 49[a]	Total	3227	959	Av. 70%[a]

[a] Positive Thorn test.

Salicylamide-treated rats showed a significant eosinopenic response in four hours. Nine of ten rats showed a fall in the eosinophil count in four hours. Five of these indicate positive adrenal function tests according to the criteria of Thorn, *et al.* (12). The average response in four hours was a 49% reduction in eosinophils and may be considered significant. The eosinopenic response was much more evident after six hours. At that time the average response was a 70% reduction in eosinophils and eight of ten rats gave a positive adrenal function test.

TABLE II.—EFFECT OF SALICYLAMIDE ON ADRENAL ASCORBIC ACID

Dose	Time, Hr.	No. of Animals	Mean Ascorbic Acid Content in mg. % ± Std. Error	Normal, %
None	0	12	313 ± 5.1	100
100 mg./Kg.	2	5	169 ± 11.0	54
100 mg./Kg.	4	5	201 ± 11.4	64
100 mg./Kg.	6	5	220 ± 13.1	70
200 mg./Kg.	2	5	133 ± 8.4	43
400 mg./Kg.	2	5	112 ± 8.4	36

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Book Notices

Spot Tests. 4th completely revised English edition. By FRITZ FEIGL (translated by Ralph E. Oesper). Vol. I: Inorganic Applications. Vol. II: Organic Applications. Elsevier Press, Inc., New York (London), 1954. Vol. I, 518 pp.; Vol. II, 436 pp. 16.5 x 23.5 cm. Price Vol. I, $6.50, Vol. II, $6.25.

The fourth edition of *Spot Tests* has been completely revised so that it now consists of two volumes instead of one, as did the previous editions. Each volume is complete in itself; both together will still be the same valuable asset to the research worker as were the former editions.

In Vol. I: Inorganic Applications retains all of the material on this subject still of value while that of no further use is deleted. In addition, about 100 pages of new, hitherto uncited tests have been added. The first few pages are devoted to general remarks about spot tests, and the first chapter on working methods, in the third edition have been revised and enlarged now to consist in this volume of two excellent chapters on these topics. The arrangement of the descriptions and directions for the individual tests remain the same retaining in this edition the same valuable style found in the former editions.

Volume II: Organic Applications consists of organic spot tests, which were previously included as a section of each of the former single-volume editions. As in the first volume, the material has been completely revised and also contains a large number of previously unpublished tests. The quantity of the new material on this subject can easily be seen in that, while this volume is not as large as Vol. I, it has practically as much material as did the third edition which contained both inorganic and organic spot tests in a single volume. Included among the 200 organic spot tests in Vol. II, are tests for many of the frequently used drugs, functional groups of medicinal chemicals, and applications of spot reactions in testing for drugs in pharmaceutical preparations including some antibiotics such as pencillin salts, streptomycin, and dihydrostreptomycin.

Both Volumes I and II of *Spot Tests* contain a wealth of information, in a well organized form, and will be of great value not only to the organic chemist or analytical chemist, but also to the pharmaceutical chemist, biochemist, and workers in other allied fields.

Medical Uses of Cortisone, Including Hydrocortisone and Corticotropin. Edited by FRANCIS D. W. LUKENS, M.D. The Blakiston Co., Inc., New York, 1954. 534 pp. Illustrated. 16 x 23.5 cm. Price $7.50.

The author's purpose to fill the needs for a compilation of the results of the investigations of the many workers on the action and uses of cortisone, hydrocortisone, and corticotropin, and to provide an authoritative compendium of sound clinical judgments concerning their use seems to have been accomplished in this book. Fifteen chapters contain a wealth of knowledge presented by twenty-nine contributors from leading institutions; the nearly two thousand references cited at the end of the chapters serve to complete the extensive coverage of the field. *Medical Uses of Cortisone, Including Hydrocortisone and Corticotropin* will prove valuable to physicians and other workers as an informative source on the use of cortisone and related hormones.

Newer Concepts of the Causes and Treatments of Diabetes Mellitus. Proceedings of the Symposium on Diabetes sponsored by the New York Diabetes Association. The National Vitamin Foundation, Inc., 1954. 181 pp. 15.5 x 23 cm. Price $2.50.

This printed report contains considerable data on various aspects of diabetes mellitus, its treatment, and various effects of the disease, as results of both clinical investigations and work on laboratory animals were discussed at the symposium. The action of insulin and indications of uses of various insulins, nutritional management of the disease, control of complications of diabetes, effects of life situations and emotions on diabetes, management of surgical infections in diabetes and lipogenesis, and hormone interrelationships in the disease are some topics on which papers were presented and discussions conducted.

Substances Naturelles de Synthèse—Préparations et Méthodes de Laboratoire. LEON VELLUZ, Editor. Masson & Cie, Paris, 1954. Vol. VIII. 157 pp. 16 x 22.5 cm. *fr.* 2,200. Vol. IX. 186 pp. 16 x 23.5 cm. *fr.* 2,280.

Volumes VIII and IX, as were the previous volumes of this excellent series of French publications, will be most welcomed by organic chemists. They,

also, were prepared by A. Allais, J. Mathieu, A. Petit, P. Poirier, and L. Velluz. The same style is followed in these volumes as in the previous ones, and the improved features of a stiff cloth-covered backing and better paper, which has been used since the fifth volume, are retained in these two volumes.

In Volume VIII are given methods of preparations for nicotinic acid, DL-tuberculo-stearic acid, dicumarol, hydrocortisone, tryptamine, and DL-tryptophane. Reduction by mixed hydrides, synthesis of saturated fatty acids and mixed hydrides are discussed.

The ninth volume contains a method of synthesis for β-indole, acetic acid, L-cysteine, DL-hydroxylysine, spermine, labeled testosterone, and thymine.

Synthesis of radio-isotope labeled natural substances are discussed with their uses and doses, and detection and reactions of them. Also discussed are decomposition methods and reagents for decomposition of these substances.

The previous six volumes of this series were reviewed in THIS JOURNAL, **40,** 534(1951); **41,** 167, 630 (1952), and **42,** 448, 643(1953).

Encyclopedia of Chemical Technology. Vol. 12. (Sabadine to Stilbestrol.) RAYMOND E. KIRK and DONALD F. OTHMER, Editors. The Interscience Publishers, Inc., New York, 1954. xvi + 955 pp. Illustrated. 20 x 27 cm. Price $30 (single copy), $25 (subscription price).

The monumental undertaking of Drs. Kirk and Othmer is approaching its final volumes. The present one, covering largely the letters, includes among other items of interest to pharmacists, a 36-page discussion on safety (with a table of incompatible chemicals); a 22-page chapter on salicylic acid; the latest information on silica and silicates, silicon, and silicones (145 pp.); and articles on shampoos and soap (22–26 pp.). You will also find items of such recent interests as seaweed colloids and stilbene dyes. No matter what the topic, the editors have always been able to win outstanding specialists for their enterprise.

Das Chlorophyll in Medizin und Kosmetik. By HANS VOGEL. Verlag Hans Carl, Nuernberg, 1954. 152 pp. 13 x 19.5 cm. Price *DM* 7.50.

In the foreword it is stated that the purpose of this book is to determine objectively the value of chlorophyll in medicine and cosmetics. That the chlorophyll industry has been permitted to expand vastly without this being first ascertained is deplored by the author.

The book consists of three parts. The first part, entitled "Chlorophyll in Medicine and Cosmetics," deals with the use of chlorophyll in such fields as dentistry, ophthalmology and dermatology in combination with the sulfa drugs and antibiotics, and as a deodorant when used medically in gynecology, in carcinoma and ear diseases, as well as in cosmetics. The occurrence of chlorophyll in nature, its chemical constitution, chlorophylls a and b, chlorophyllase other chemical aspects, and the function of chlorophyll in nature are some of the topics covered in the second part, "Chlorophyll in Chemistry and Physiology." Commercial aspects of production of chlorophyll and its derivatives, and methods of analysis, including spectrophotometric, are discussed in the last part, "The Production of Chlorophyll and Its Derivatives." A bibliography of the literature reported is given.

Méthodes et Réactions de l'Analyse Organique. Vol. III. (Réactions Colorées et Fluorescences.) LEON VELLUZ, Editor. Masson & Cie, Paris, 1954. 298 pp. 16.5 x 25 cm. *fr.* 2,750.

This volume is the last of three volumes and was also prepared by Maurice Pesez and Pierre Poirier in collaboration with J. Mathieu, A. Petit, and J. Wohlgemuth. The first two volumes were reviewed in THIS JOURNAL, **41,** 457 (1952), and **42,** 644(1953), respectively.

The text is divided under the following section headings: (1) Oxidation-reduction, (2) Metallic and metaloid reagents, (3) Diazotation-Copulation, (4) Imines, Aldehydes-Amines, (5) Indophenol, Phenolamine Reactions, (6) Aryl-Methanes, Aldehyde-phenol Reactions, (7) Heterocycles, (8) Condensation of Methylene, and (9) Halochrome-Halofluorine. As in the previous volumes, general discussions and application of the procedures, an extensive bibliography, and tabulated data are included for each topic. A general index and table of principal reactions are given.

Thérapeutiques Récentes—Antibiotiques, Radio-Isotopes, A.C.T.H., Cortisone, Resines Échangeuses de Cations, Isoniazide, Hyperthyroidies. By R. TURPIN, Y. CHABERT R. FAUVERT, G. BROUET, CL. LAROCHE, H. BRICAIRE, GILBERT DREYFUS, S. BLONDIN, M. ZARA, and J.-C. SAVOIE. Masson & Cie, Paris, 1954. 152 pp. 17 x 22.5 cm. *fr.* 800.

Since 1947 the "Conferences D'Actualities Pratiques" de la Faculté de Médecine de Paris has met annually for the purpose of discussing the latest attainments in the fields of medicine, biology, radiology, and surgery, and the topics covered at the past conference are reported in this book. Aspects of antibiotic treatment, with some antibiotics being individually discussed, are given here. The medical applications of I-131 was the subject of the section on radioisotopes. Present indications of cortisone, hydrocortisone, and A.C.T.H. in such instances as Addison's disease, glandular disorders, rheumatic cases, ophthalmology, and allergies are well covered. The cation-exchange resins as new therapeutic agents and their actions, indications, and toxicity are discussed.

Clinical results in the use of the recent antitubercular isoniazid are given. Under hyperthyroidism the subjects of the hypophysis, thyroid glands and the physio-pathology diagnosis, and various types of treatment of hyperthyroid conditions, are discussed.

Arrest of Bleeding—Physiology, Pharmacology Pathology. By JACQUES ROSKAM, CHARLES C THOMAS, Publisher, Springfield, Ill., 1954. 71 pp. 14.5 x 22 cm. Paper cover. Price $2.75.

The author is Professor of Internal Medicine at the University of Liége, and his book has been translated from the French. It presents an analytical and physiological study of spontaneous hemo-

stasis, a pharmacological investigation of hemostatic agents, and a discussion of symptoms, prophylaxis and therapy of hemorrhagic diseases and thrombosis. The book is valuable because it is largely based on the author's own experimental studies which have not heretofore been published in English. Among the hemostatic agents tested are adrenochrome, the sympathomimetic amines, the naphthoquinones and quinones, and various sympatholytic substances.

The Macmillan Medical Dictionary. Edited by Sir Cecil Wakeley, Bt. The Macmillan Company, New York, 1954. vii + 471 pp. 26 x 18 cm. Price $6.95.

The Faber Medical Dictionary appearing in the U. S. under the disguise of *Macmillan Medical Dictionary* is distinguished by short pointed definitions addressed primarily to physicians, pharmacists, and nurses. Thus, it serves as a perfect complement to Black's, the other outstanding English publication in this field, whose first concern is the lay public and which excels in long discursive essays on major topics. The information supplied is as up to date as may be reasonably expected from a work of this kind: e. g., aureomycin (1952) is listed while tetracycline (1953) is not to be found. The lack of unnecessary cumbersome cross-references facilitates the use of the dictionary. The illustrative material is rather scanty. It might be well to caution any prospective users that, of course English spelling (haem-instead of hem-) is used in the relatively few cases where it differs from the American usage.

BOOKS RECEIVED

Biochemistry of Cancer. 2nd, revised ed. By JESSE GREENSTEIN. Academic Press, Inc., Publishers, New York, N. Y., 1954. 653 pp. 16.5 x 23 cm. Price $12.

The Practice of Modern Perfumery. By PAUL JELLENEK. Interscience Publishers, Inc., New York, 1954. x + 219 pp. 22 x 14.5 cm. Price $4.75.

The Macmillan Medical Dictionary. Edited by SIR CECIL WAKELEY, BT. The Macmillan Company, New York, 1954. vii + 471 pp. 26 x 18 cm. Price $6.95.

History of Indian Pharmacy. Vol. 1, 2nd ed. By G. P. SRIVASTAVA, Pindars Ltd., Calcutta, 1954. xvi + 277 pp. 22.5 x 14 cm. Price $2.50.

The Infra-Red Spectra of Complex Molecules. By L. J. BELLAMY. John Wiley & Sons, Inc., New York, 1954. xvii + 323 pp. 22.5 x 14 cm. Price $7.

Legal Medicine, Pathology and Toxicology. 2nd ed. By THOMAS A. GONZALES, MORGAN VANCE, MILTON HELPERN, and CHARLES J. UMBERGER. Appleton-Century-Crofts, Inc., New York, 1954. xii + 1349 pp. 25 x 17 cm. Price $22.

The Vitamins—Chemistry, Physiology, Pathology. Vol. II. W. H. SEBRELL, JR., and ROBERT S. HARRIS, Editors. Academic Press, Inc., New York, 1954. xiii + 766 pp. 9.25 x 6 cm. Price $16.50.

Practical Physiological Chemistry. 13th ed. By PHILIP B. HAWK, BERNARD L. OSER, and WILLIAM H. SUMMERSON. The Blakiston Co., Inc., New York, 1954. xvi + 1439 pp. 23.5 x 16 cm. Price $12.

Bentley's Textbook of Pharmaceutics. 6th ed. Revised by HAROLD DAVIS, with the collaboration of M. W. PARTRIDGE, and C. L. SARGENT, with contributions by W. A. BOOM, H. A. TURNER, and M. TURNER, Bailiere, Tindall, and Cox, London. xiii + 1078 pp. 22.5 x 14.5 cm. Price $10.

Chemical Specificity in Biological Interactions. Edited by F. R. N. GURD. Academic Press, Inc., New York, 1954. xv + 234 pp. 23 x 16 cm. Price $6.

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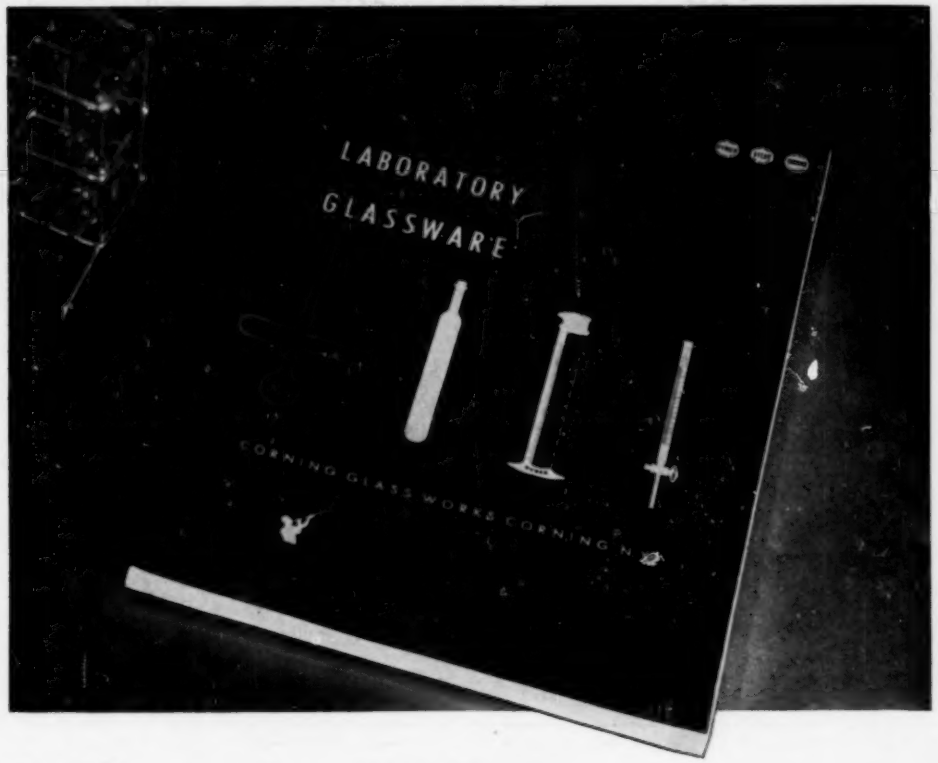

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